

## **MARKERS FOR DETECTION OF GASTRIC CANCER**

### **Related Application**

This application claims priority under 35 U.S.C. 119 to United States Provisional Patent Application Serial No: 60/487,906, filed July 17, 2003, titled "Markers for Detection of Gastric Cancer," listing Parry John Guilford as inventor. The above application is herein incorporated fully by reference.

### **Field of the Invention**

This invention relates to detection of cancer. Specifically, this invention relates to the use of genetic and/or protein markers for detection of cancer, and more particularly to the use of genetic and/or protein markers for detection of gastric cancer.

## **BACKGROUND**

Survival of cancer patients is greatly enhanced when the cancer is detected and treated early. In the case of gastric cancer, patients diagnosed with early stage disease have 5-year survival rates of 90%, compared to approximately 10% for patients diagnosed with advanced disease. However, the vast majority of gastric cancer patients currently present with advanced disease. Therefore, developments that lead to early diagnosis of gastric cancer can lead to an improved prognosis for the patients.

Identification of specific cancer-associated markers in biological samples, including body fluids, for example, blood, urine, peritoneal washes and stool extracts can provide a valuable approach for the early diagnosis of cancer, leading to early treatment and improved prognosis. Specific cancer markers also can provide a means for monitoring disease progression, enabling the efficacy of surgical, radiotherapeutic and chemotherapeutic treatments to be tracked. However, for a number of major cancers, the available markers suffer from insufficient sensitivity and specificity. For example, the most frequently used markers for gastric cancer, ca19-9, ca72-4 and chorioembryonic antigen (CEA) detect only about 15-50% of gastric tumors of any stage, declining to approximately 2-11% for early stage disease. Thus, there is a very high frequency of false negative tests that can lead patients and health care practitioners to believe that no disease exists, whereas in fact, the patient may have

severe cancer that needs immediate attention. Moreover, these markers can give false positive signals in up to 1/3 of individuals affected by benign gastric disease.

### SUMMARY OF THE INVENTION

Thus, there is an acute need for better methods for detecting the presence of cancer. Aspects of this invention provide methods, compositions and devices that can provide for detection of early stage cancer, and decreasing the frequency of false positives and false negative test results.

In certain embodiments, molecular analysis can be used to identify genes that are over-expressed in gastric tumor tissue compared to non-malignant gastric tissue. Such analyses include microarray and quantitative polymerase chain reaction (qPCR) methods. Cancer genes and proteins encoded by those genes are herein termed gastric tumor markers (GTM). It is to be understood that the term GTM does not require that the marker be specific only for gastric tumors. Rather, expression of GTM can be increased in other types of tumors, including malignant or non-malignant tumors, including gastric, bladder, colorectal, pancreatic, ovarian, skin (e.g., melanomas), liver, esophageal, endometrial and brain cancers, among others. It should be understood, however that the term GTM does not include prior art markers, ca19-9, ca72-4 and CEA. Some GTM are sufficiently over-expressed to be diagnostic of gastric cancer with a high degree of reliability, and in other cases, over-expression of two or more GTM can provide reliable diagnosis of gastric cancer.

In certain embodiments, microarray methods can be used to detect patterns of over-expression of one or more genes associated with cancer.

In other embodiments, quantitative polymerase chain reaction (qPCR) can be used to identify the presence of markers over expressed in tumor or other biological samples.

Some of the embodiments of GTM detection disclosed herein are over expressed in a highly selective fashion in tumor cells and little, if at all, in non-tumor cells, permitting sensitive and accurate detection of cancer with measurement of only one over expressed GTM. In other embodiments, over-expression of two, three or more GTM can be detected in a sample and can provide greater certainty of diagnosis.

Selected genes that encode proteins can be secreted by or cleaved from the cell. These proteins, either alone or in combination with each other, have utility as serum or body fluid markers for the diagnosis of gastric cancer or as markers for

monitoring the progression of established disease. Detection of protein markers can be carried out using methods known in the art, and include the use of monoclonal antibodies, polyclonal antisera and the like.

### BRIEF DESCRIPTION OF THE FIGURES

This invention is described with reference to specific embodiments thereof and with reference to the figures, in which:

Figure 1 depicts a table of markers and oligonucleotide sequences of markers for gastric cancer of this invention.

Figure 2 depicts a table of results obtained of studies carried out using microarray methods.

Figure 3 depicts a table of results obtained of studies carried out using quantitative PCR.

Figures 4a – 4d depict relationships between log<sub>2</sub> fold results obtained using array and qPCR methods, in which the data is centered on the median normal for four gastric cancer markers. Grey squares correspond to non-malignant (“normal”) samples and black triangles to tumor samples. Figure 4a: ASPN. Figure 4b: SPP1. Figure 4c: SPARC. Figure 4d: MMP12.

Figures 5a-5w depict histograms showing the relative frequency vs. log<sub>2</sub> fold change data obtained from quantitative PCR studies of various tumor markers. Figure 5a: ASPN; Figure 5b: CST1,2 & 4; Figure 5c: CSPG2; Figure 5d: IGFBP7; Figure 5e: INHBA; Figure 5f: LOXL2; Figure 5g: LUM; Figure 5h: SFRP4; Figure 5i: SPARC; Figure 5j: SPP1; Figure 5k: THBS2; Figure 5l: TIMP1; Figure 5m: adlcan; Figure 5n: PRS11; Figure 5o: ASAH1; Figure 5p: SFRP2; Figure 5q: GGH; Figure 5r: MMP12; Figure 5s: KLK10; Figure 5t: LEPRE1; Figure 5u: TG; Figure 5v: EFEMP2 and Figure 5w: TGFBI.

Figure 6 is a histogram showing the number of markers with a higher expression than the 95<sup>th</sup> percentile of the median normal expression. Results are based on qPCR data and are shown separately for each tumor sample.

Figures 7a- 7c depicts graphs that show relative log<sub>2</sub> expression of the markers in individual tumor samples and non-malignant samples compared to the expression of the gene for the tumor marker, CEA. CEA is the serum marker currently most used to monitor progression of gastric cancer.

Figure 8 shows a table that complements Figure 3. Figure 8 summarizes expression levels determined by qPCR for the candidate tumor markers, but using the paired data (i.e., tumor ("T") and non-malignant ("N") samples from the same individual) to provide a T:N ratio. Figure 8 also includes additional markers not included in Figure 3, namely MMP2, CGR11, TGFB1, PCSK5, SERPINB5, SERPINH1. For comparison, the expression level of the established serum marker gene, CEACAM5 (CEA), is also shown. 27 of the 29 markers have a median T:N difference greater than or equal to CEA. Further, compared to CEA, 29/29 of the markers have a higher percentage of paired samples in which the expression in the tumor sample exceeds the expression in the normal sample. Three markers, CST1,2,44, ASPN and SFRP4 showed 100% discrimination between the paired tumor and normal samples. The gene sequences of these markers, and the location of the primers and probes used to detect them, are shown herein.

Figures 9a – 9d depict individual and median T:N fold change data for 29 gastric cancer markers in 40 patients with paired samples.

Figures 10a – 10ad depict graphs of tumor stage and log2 fold change in expression of CEA and other GTM of this invention. Figure 10a: adlcan; Figure 10b: ASPN; Figure 10c: CSPG2; Figure 10d: CST1,2,4; Figure 10e: EFEMP2; Figure 10f: GGF; Figure 10g: INHBA; Figure 10h: IGFBP7; Figure 10i: KLK10; Figure 10j: LEPRE1; Figure 10k: LUM; Figure 10l: LOXL2; Figure 10m: MMP12; Figure 10n: TIMP1; Figure 10o: ASAH1; Figure 10p: SPP1; Figure 10q: SFRP2; Figure 10r: SFRP4; Figure 10s: SPARC; Figure 10t: PRSS11; Figure 10u: THBS2; Figure 10v: TG; Figure 10w: TGFB1; Figure 10x: CGR11; Figure 10y: SERPINH1; Figure 10z: MMP2; Figure 10aa: PCSK5; Figure 10ab: SERPINB5; Figure 10ac: TGFB1 and Figure 10ad: CEA (CEACAM5).

Figures 11a – 11ad depict graphs of tumor type (diffuse (D) or intestinal (I)) and log2 fold change in expression 29 GTM of this invention and CEA. Figure 11a: adlcan; Figure 11b: ASPN; Figure 11c: CSPG2; Figure 11d: CST1,2,4; Figure 11e: EFEMP2; Figure 11f: GGF; Figure 11g: INHBA; Figure 11h: IGFBP7; Figure 11i: KLK10; Figure 11j: LEPRE1; Figure 11k: LUM; Figure 11l: LOXL2; Figure 11m: MMP12; Figure 11n: TIMP1; Figure 11o: ASAH1; Figure 11p: SPP1; Figure 11q: SFRP2; Figure 11r: SFRP4; Figure 11s: SPARC; Figure 11t: PRSS11; Figure 11u: THBS2; Figure 11v: TG; Figure 11w: TGFB1; Figure 11x: CGR11; Figure 11y:



SERPINH1; Figure 11z: MMP2; Figure 11aa: PCSK5; Figure 11ab:SERPINB5; Figure 11ac: TGFBI and Figure 11ad: CEA (CBACAM5).

Figure 12 depicts a three-dimensional graph showing 3 markers, SERPINH1, CST1,2,4 and INHBA, in a series of gastric tumor samples and non-malignant gastric samples.

Figure 13 depicts a table that shows the effect of multiple markers on the ability to accurately discriminate between tumor tissue and non-malignant tissue. The table has been derived from normal distributions derived from qPCR data.

Figure 14 is a Western blot of 4 tumor markers derived from tumor and non-tumor tissue.

Figure 15 is a Western blot of the tumor marker SPARC in gastric tumor tissue and in serum.

Figure 16 is an immunoblot depicting cystatin SN in the supernatant of a gastric cell line, AGS.

## DETAILED DESCRIPTION

### Definitions

Before describing embodiments of the invention in detail, it will be useful to provide some definitions of terms as used herein.

The term "GTM" or "gastric tumor marker" or "GTM family member" means a gene, gene fragment, RNA, RNA fragment, protein or protein fragment related or other identifying molecule associated with gastric cancer that does not include molecules that are known in the prior art to be associated with gastric cancer, ca19-9, ca72-4 and CEA. Examples of GTMs are included herein below.

The term "marker" means a molecule that is associated quantitatively or qualitatively with the presence of a biological phenomenon. Examples of "markers" are GTMs, however, "markers" also includes metabolites, byproducts, whether related directly or indirectly to a mechanism underlying a condition.

The term "qPCR" means quantitative polymerase chain reaction.

The term "expression" includes production of mRNA from a gene or portion of a gene, and includes the production of a protein encoded by an RNA or gene or portion of a gene, and includes appearance of a detection material associated with expression. For example, the binding of a binding ligand, such as an antibody, to a gene or other oligonucleotide, a protein or a protein fragment and the visualization of

the binding ligand is included within the scope of the term "expression." Thus, increased density of a spot on an immunoblot, such as a Western blot, is included within the term "expression" of the underlying biological molecule.

The term "CPN2" means human carboxypeptidase N, polypeptide 2, 83 kDa chain; and carboxypeptidase N.

The term "HAPLN4" means human hyaluronan glycoprotein link protein 4.

The term "MMP12" means human matrix metalloproteinase 12.

The term "INHBA" means human inhibin, beta A (also includes activin A, activin AB or alpha polypeptide).

The term "IGFBP7" means human insulin-like growth factor 7.

The term "GGH" means human gamma-glutamyl hydrolase (also known as conjugase, folylpolyglutaminyl hydrolase).

The term "LEPRE1" means human leucine proline-enriched proteoglycan (also known as leprecan 1).

The term "CST4" means human cystatin S.

The term "SFRP4" means human secreted frizzled-related protein 4.

The term "ASPN" means human asporin (also known as LRR class 1).

The term "CGREF1" or "CGR11" means human cell growth regulator with EF hand domain 1.

The term "KLK" means either human kallikrein 10, variant 1 or human kallikrein 10, variant 2, or both, unless specified otherwise.

The term "TIMP1" means human tissue inhibitor of metalloproteinase 1 (also known as erythroid potentiating activity or collagenase inhibitor).

The term "SPARC" means human secreted protein, acidic, cysteine-rich (also known as osteonectin).

The term "TGFB1" means human transforming growth factor, beta-induced, 68kDa.

The term "EFEMP2" means human EGF-containing fibulin-like extracellular matrix protein 2.

The term "LUM" means human lumican.

The term "SNN" means human stannin.

The term "SPP1" means human secreted phosphoprotein 1 (also known as osteopontin, or bone sialoprotein I, or early T-lymphocyte activation 1).

The term "CSPG2" means human chondroitin sulfate proteoglycan 2 (also known as versican).

The term "ASAH1" means human N-acylsphingosine amidohydrolase, variant 1, or N-acylsphingosine amidohydrolase, variant 2, or both N-acylsphingosine amidohydrolase variants 1 and 2 (also known as acid ceramidase 1, variants 1 and 2).

The term "PRSS11" means human protease, serine, 11 (also known as IGF binding serine protease).

The term "SFRP2" means human secreted frizzled-related protein 2.

The term "PLA2G12B" means human phospholipase A2, group XIIB.

The term "SPON2" means human spondin 2, extracellular matrix protein.

The term "OLFM1" means human olfactomedin 1.

The term "TSRC1" means human thrombospondin repeat containing 1.

The term "THBS2" means human thrombospondin 2.

The term "adlcan" means DKFZp564I1922.

The term "CST2" means human cystatin SA.

The term "CST1" means human cystatin SN.

The term "LOXL2" means human lysyl oxidase-like enzyme 2.

The term "TG" means human thyroglobulin.

The term "TGFB1" means human transforming growth factor, beta1 .

The term "SERPINH1" means human serine or cysteine proteinase inhibitor clade H (also known as heat shock protein 47, member 1, or collagen binding protein 1).

The term "SERPINB5" means human serine or cysteine proteinase inhibitor, clade B (also known as ovalbumin, member 5).

The term "CEACAM5" or "CEA" means human carcinoembryonic antigen-related cell adhesion molecule 5.

The term "MMP2" means human matrix metalloproteinase 2 (also known as gelatinase A, or 72 kDa gelatinase, or 72 kDa type IV collagenase).

The term "PCSK5" means human proprotein convertase subtilisin/kexin type 5.

It is to be understood that the above terms may refer to protein, DNA sequence and/or RNA sequence. It is also to be understood that the above terms also refer to non-human proteins, DNA and/or RNA having the same sequences as depicted herein.

**Description of Embodiments of the Invention**

Markers for detection and evaluation of tumors including gastric cancer are provided that have a greater reliability in detecting gastric cancer than prior art markers. By the term "reliability" we include the absence of false positives and/or false negatives. Thus, with higher reliability of a marker, fewer false positives and/or false negatives are associated with diagnoses made using that marker. Therefore, in certain embodiments, markers are provided that permit detection of gastric cancer with reliability greater than the reliability of prior art markers of about 50%. In other embodiments, markers are provided that have reliability greater than about 70%; in other embodiments, greater than about 73%, in still other embodiments, greater than about 80%, in yet further embodiments, greater than about 90%, in still others, greater than about 95%, in yet further embodiments greater than about 98%, and in certain embodiments, about 100% reliability.

Thus, we have surprisingly found numerous genes and proteins whose presence is associated with gastric tumors. Detection of gene products (e.g., oligonucleotides such as mRNA) and proteins and peptides translated from such oligonucleotides therefore can be used to diagnose tumors, such as gastric tumors. Array analysis of samples taken from patients with gastric tumors and from non-malignant tissues of the same subjects has led us to the surprising discovery that in many gastric tumors, specific patterns of over-expression of certain genes are associated with the disease.

Cancer markers can also be detected using antibodies raised against cancer markers.

By analyzing the presence and amounts of expression of a plurality of cancer markers can thus increase the sensitivity of diagnosis while decreasing the frequency of false positive and/or false negative results.

**General Approaches to Cancer Detection**

The following approaches are non-limiting methods that can be used to detect cancer including gastric cancer using GTM family members.

- Microarray approaches using oligonucleotide probes selective for products of GTM genes.

- Real-time quantitative PCR (qPCR) on tumor samples and normal samples using marker specific primers and probes.
- Enzyme-linked immunological assays (ELISA).
- Immunohistochemistry using anti-marker antibodies on gastric tumors and lymph node metastases.
- Immunohistochemistry using anti-marker antibodies on other tumors including but not limited to colorectal, pancreatic, ovarian, melanoma, liver, esophageal, bladder, endometrial, and brain.
- Immunodetection of marker family members in sera from gastric cancer patients taken before and after surgery to remove the tumor.
- Immunodetection of marker family members in sera from healthy individuals and individuals with non-malignant diseases such as gastritis, ulceration, gastric metaplasia and dysplasia.
- Immunodetection of marker family members in patients with other cancers including but not limited to colorectal, pancreatic, ovarian, melanoma, liver, oesophageal, bladder, endometrial, and brain.
- Detection of markers in body fluids, including serum, lymph, peritoneal fluid, cerebrospinal fluid, synovial fluid and the like.
- Immunodetection of marker family members in gastric fluid, peritoneal washes, urine and stool from gastric cancer patients. Using array methods and/or qPCR.
- Analysis of array or qPCR data using computers. Primary data is collected and fold change analysis is performed by comparison of levels of gastric tumor gene expression with expression of the same genes in non-tumor tissue. A threshold for concluding that expression is increased is provided (e.g., 1.5 x increase, 2-fold increase, and in alternative embodiments, 3-fold increase, 4-fold increase or 5-fold increase). It can be appreciated that other thresholds for concluding that increased expression has occurred can be selected without departing from the scope of this invention. Further analysis of tumor gene expression includes matching those genes exhibiting increased expression with expression profiles of known gastric tumors to provide diagnosis of tumors.

In certain aspects, this invention provides methods for detecting cancer, comprising:

- (a) providing a biological sample; and
- (b) detecting the over expression of a GTM family member in said sample.

In other aspects, the invention includes a step of detecting over expression of GTM mRNA.

In other aspects, the invention includes a step of detecting over expression of a GTM protein.

In yet further aspects, the invention includes a step of detecting over-expression of a GTM peptide.

In still further aspects, the invention includes a device for detecting a GTM, comprising:

- a substrate having a GTM capture reagent thereon; and
- a detector associated with said substrate, said detector capable of detecting a GTM associated with said capture reagent, wherein the capture reagent includes an oligonucleotide or an antibody.

Additional aspects include kits for detecting cancer, comprising:

- a substrate;
- a GTM capture reagent, including one or more of a GTM-specific oligonucleotide and a GTM-specific antibody; and
- instructions for use.

Yet further aspects of the invention include method for detecting a GTM using qPCR, comprising:

- a forward primer specific for said GTM;
- a reverse primer specific for said GTM;
- PCR reagents;
- a reaction vial; and
- instructions for use.

Additional aspects of this invention comprise a kit for detecting the presence of a GTM protein or peptide, comprising:

- a substrate having a capture agent for said GTM protein or peptide;
  - an antibody specific for said GTM protein or peptide;
  - a reagent capable of labeling bound antibody for said GTM protein or peptide;
- and
- instructions for use.

Additional aspects of this invention include a method for manufacturing a monoclonal antibody, comprising the steps of:

In yet further aspects, this invention includes a method for detecting gastric cancer, comprising the steps of:

providing a sample from a patient suspected of having gastric cancer;  
measuring the presence of a GTM protein using an ELISA method.

As described herein, detection of tumors can be accomplished by measuring expression of one or more tumor-specific markers. We have unexpectedly found that the association between increased expression of GTMs and the presence of diagnosed gastric cancer is extremely high. The least significant association detected had a p value of about  $1.6 \times 10^{-6}$ . Many of the associations were significant at p values of less than  $10^{-20}$ . With such a high significance, it may not be necessary to detect increased expression in more than one GTM. However, the redundancy in the GTMs of this invention can permit detection of gastric cancers with an increased reliability.

The methods provided herein also include assays of high sensitivity. qPCR is extremely sensitive, and can be used to detect gene products in very low copy number (e.g., 1 – 100) in a sample. With such sensitivity, very early detection of events that are associated with gastric cancer is made possible.

## Methods

The following general methods were used to evaluate the suitability of various approaches to molecular identification of markers associated with gastric tumors.

### Tumor Collection

Gastric tumor samples and non-malignant gastric tissues were collected from surgical specimens resected at Seoul National University Hospital, Korea and Dunedin Hospital, New Zealand. Diagnosis of gastric cancer was made on the basis of symptoms, physical findings and histological examination of tissues.

### RNA Extraction

In some embodiments, expression of genes associated with gastric tumors was analyzed by determining the changes in RNA from samples taken from tumors. Frozen surgical specimens were embedded in OCT medium. 60 $\mu$ m sections were sliced from the tissue blocks using a microtome, homogenized in a TriReagent: water

(3:1) mix, then chloroform extracted. Total RNA was then purified from the aqueous phase using the RNeasy<sup>TM</sup> procedure (Qiagen). RNA was also extracted from 16 cancer cell lines and pooled to serve as a reference RNA.

#### **Microarray Slide Preparation**

Epoxy coated glass slides were obtained from MWG Biotech AG, Ebersberg, Germany) and were printed with ~30,000 50mer oligonucleotides using a Gene Machines microarraying robot, according to the manufacturer's protocol. Reference numbers (MWG oligo #) for relevant oligonucleotides, and the NCBI mRNA and protein reference sequences are shown in Figure 2. Full DNA sequences of the GTM of this invention are shown herein below.

#### **RNA labeling and Hybridization**

cDNA was transcribed from 10µg total RNA using Superscript II reverse transcriptase (Invitrogen) in reactions containing 5-(3-aminoallyl)- 2' deoxyuridine – 5'-triphosphate. The reaction was then de-ionized in a Microcon column before being incubated with Cy3 or Cy5 in bicarbonate buffer for 1 hour at room temperature. Unincorporated dyes were removed using a Qiaquick column (Qiagen) and the sample concentrated to 15ul in a SpeedVac. Cy3 and Cy5 labeled cDNAs were then mixed with Ambion ULTRAhyb buffer, denatured at 100°C for 2 minutes and hybridized to the microarray slides in hybridization chambers at 42°C for 16 hours. The slides were then washed and scanned twice in an Axon 4000A scanner at two power settings to yield primary fluorescence data on gene expression.

#### **Normalization Procedure**

To compare expression of cancer genes from tumors and non-cancerous tissues, median fluorescence intensities detected by Genepix<sup>TM</sup> software were corrected by subtraction of the local background fluorescence intensities. Spots with a background corrected intensity of less than zero were excluded. To facilitate normalization, intensity ratios and overall spot intensities were log-transformed. Log-transformed intensity ratios were corrected for dye and spatial bias using local regression implemented in the LOCFIT<sup>TM</sup> package. Log-transformed intensity ratios were regressed simultaneously with respect to overall spot intensity and location. The



residuals of the local regression provided the corrected log-fold changes. For quality control, ratios of each normalized microarray were plotted with respect to spot intensity and localization. The plots were subsequently visually inspected for possible remaining artifacts. Additionally, an analysis of variance (ANOVA) model was applied for the detection of pin-tip bias. All results and parameters of the normalization were inserted into a Postgres-database for statistical analysis.

### **Statistical Analysis**

Statistically significant changes in gene expression in tumor samples vs. normal tissues were identified by measured fold changes between arrays. To accomplish this,  $\log_2$  (ratios) were scaled to have the same overall standard deviation per array. This standardization procedure reduced the average within-tissue class variability. The  $\log_2$  (ratios) were further shifted to have a median value of zero for each oligonucleotide to facilitate visual inspection of results. A rank-test based on fold changes was then used to improve the noise robustness. This test consisted of two steps: (i) calculation of the rank of fold change (Rfc) within arrays and ii) subtraction of the median (Rfc) for normal tissue from the median(Rfc) for tumor tissue. The difference of both median ranks defines the score of the fold change rank presented in Figure 2. Two additional statistical tests were also performed on this standardized data: 1) Two sample student's t-test, with and without the Bonferroni adjustment and 2) the Wilcoxon test.

### **Statistical Analysis of Marker Combinations**

To determine the value of using combinations of two or three of the markers to discriminate between tumor and non-malignant samples, the qPCR data from 40 paired samples (tumor and non-malignant samples from the same patient) were subjected to the following analysis. Normal distributions for the non-malignant and tumor samples were generated using the sample means and standard deviations. The probability that values taken from the tumor expression data would exceed a defined threshold (e.g., greater than 50%, 70%, 73%, 80%, 90%, 95%, 98%, 99% or 100%) in the non-malignant distribution was then determined (i.e., sensitivity). For combinations of markers, the probability that at least one marker exceeded the threshold was determined.

name	symbol	Applied BioSystems "assay on demand" assay #	forward primer	Seq ID No.	reverse primer	Seq ID No.	probe	Seq ID No.
asporin (tr class 1)	ASPN		AATACAAAGGAGCAATTCAGGA	1	TGCTTCGCAATTCGATATGGA	23	TTGGAATATGATGCAAGGCTCTTATATATG	45
chondrin sulfate proteoglycan 2 (versican)	CSF2		GGCAGTGATGATGCTTCC	2	TCTTGCAATTTCTACACAGGG	24	AGCAACAGTTTCTTCCGCGAC	46
crystallin SN, SA & S	CSTL 2, 4		AGTCCAGCCCACTTGA	3	GGGAAGTCTGTAGATCTGAAAGA	25	AGCCAGACTGCAGAGGAAACAGTTGTC	47
gamma-glutamyl hydrolase	GGM		GTGGCATGCGCTGAA	4	TGACAGCAACACTCTAGTAGAAGA	26	TTCACTGAGGCTCAATTTCACAGCGAGAT	48
insulin-like growth factor binding protein 7	IGFBP7		CAGGTACAGAGGGACCC	5	TCAAGCTTCAAGTACACCTGGG	27	AGCAAGGCTCTTCAATAGTAGAGGCTC	49
kalikrein 10	KLK10		ACACATGATATGCTGGACTGG	6	GAGAGATGCTCTTGGAGGGT	28	CTTCCAGAGTCACTCTGGAGGCTC	50
leucine proline-enriched proteoglycan 1 (leprecan 1)	LEPRE1		CTTGAGTACACCGCTGACTTTC	7	CGGTGACACAGTCTTCTCTACAG	29	CCATCAGATCATTAATACATCGAGTCTCA	51
lumican	LUM		GATCTTGTCATATAGTGCATCTGC	8	CCATTCATTCGAGGAGAGAGA	30	TAGGATTCACCACTTTGCCAAAATGAGTCTAA	52
lysyl oxidase-like 2	LOXL2		AGGCCAGCTTCTGCTTGA	9	CCCTGATCCCGAGTTG	31	CGTATTCTCTGGAATGCTCTCTTCACTCTG	53
matrix metalloproteinase 12	MMP12		GGCTCTCTGCTGATGATAGT	10	AGTGACAGCATCAAACTCAATTG	32	TCAGTCCCTGTATGGAGAGCCCAAGAGAGA	54
metalloproteinase inhibitor 1	THP1		CCAGACCACTTATACAGGG	11	GGAGCTGTGGAAGTATCCGC	33	CAGATGACCAAGATGTATATAAGGTTCCAC	55
n-acylserine amidohydrolase	ASAH1		CGCAGACGCTTCCAAA	12	ACAGGACATCATCATGCTTTCAA	34	TGCTTGAAAGCCACACAGCAGAGATA	56
secreted frizzled-related protein 2	SFRP2		CGCTAGCAGGACCACT	13	TTTTCAGGCTTCACTACTTTT	35	CTTCCAGGACCGGAGGAGCTC	57
secreted protein, acidic, cysteine rich	SPARC		TCTTCCCTGTACACTGGCAGTTC	14	GAAAAGCGGGTGGTGA	36	TGGACCGACGACCACTTTAGGG	58
serine protease 11 (IGF binding)	PRSS11		TCGGAGGCGCGTTAGTAA	15	AAGGAGATTCCAGCTGTCTTTC	37	AGTGTATTTCATCAATCACTTCACCGTCCAGG	59
thrombospondin 2	THBS2		TGGAAGGACTACACGGCTATAG	16	TAGSTTGGTCATAGTAGTCTCTGAGT	38	AGGCCAGACCGGCTACATTCAGCTC	60
thromboglobulin	TG		GAGGCTCTCTCCAGTTCAA	17	TGTAAAGGCTCCACTTCAAT	39	TCTGGGAGATTCCGATGTCGCCAAGA	61
human cell growth regulator with EF hand domain 1	CGR11		CTGCCAGCCCTTCA	18	TTCTGTCTTCTAGTCTCTTAGG	40	CCAGGCGAGGAGGAGCTCGG	62
human serine or cysteine proteinase inhibitor clade B	SERPINB5		TCACGATTTTCCAGTAA	19	AAGCGAATTTCTAGTTGCA	41	TGACTCCAGGCGCGCGCATGCA	63
transforming growth factor beta	TGFB1		GTCATGTCATCCACCATGTT	20	TCTGCAGTGTATCCCTCTTT	42	CAGGCTTCCAGCCACAGAGCTCAGG	64
human proprotein convertase subtilisin/kexin type 5	PCSK5		AAATCTTTTCCGGAATTC	21	AGTCTGGGCTGTGAAATAC	43	ACAGATGTAGAGGATGGTTAAGCTTCCA	65
matrix metalloproteinase 2	MMP2		TTGATGGCATCCCTCAGATC	22	TGTCAGTGGCGCTCAGCT	44	TTCAAGGACCGGTTTCTTTTGGG	66
human serine or cysteine proteinase inhibitor clade H	SERPINH1	HS00241844 ml						
adipon	.	HS00377849 ml						
egf-containing fibulin-like extracellular matrix protein 2	EFEMP2	HS00213545 ml						
secreted frizzled-related protein 4	SFRP4	HS00180066 ml						
inhibin beta A chain	INHBA	HS00170103 ml						
osteopontin	SPP1	HS00167093 ml						
transforming growth factor beta-induced	TGFB1	HS00165908 ml						

Figure 1

Table 1

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### Quantitative Real-Time PCR

In other embodiments, real-time, or quantitative PCR (qPCR) can be used for absolute or relative quantitation of PCR template copy number. Taqman<sup>TM</sup> probe and primer sets were designed using Primer Express V 2.0<sup>TM</sup> (Applied Biosystems). Where possible, all potential splice variants were included in the resulting amplicon, with amplicon preference given to regions covered by the MWG-Biotech-derived microarray oligonucleotide. Alternatively, if the target gene was represented by an Assay-on-Demand<sup>TM</sup> expression assay (Applied Biosystems) covering the desired amplicons, these were used. The name of the gene, symbol, the Applied Biosystems "assay on demand" number, forward primer, reverse primer and probe sequence used for qPCR are shown in Table 1 and in Figure 1. In the in-house designed assays, primer concentration was titrated using a SYBR green labeling protocol and cDNA made from the reference RNA. Amplification was carried out on an ABI Prism<sup>TM</sup> 7000 sequence detection system under standard cycling conditions. When single amplification products were observed in the dissociation curves, standard curves were generated over a 625-fold concentration range using optimal primer concentrations and 5'FAM - 3'TAMRA phosphate Taqman<sup>TM</sup> probe (Proligo) at a final concentration of 250nM. Assays giving standard curves with regression coefficients over 0.98 were used in subsequent assays. It can be appreciated that in other embodiments, regression coefficients need not be as high. Rather, any standard curve can be used so long as the regression coefficients are sufficiently high to permit statistically significant determination of differences in expression. Such regression coefficients may be above about 0.7, above about 0.8, above about 0.9 or above about 0.95 in alternative embodiments.

Assays were performed over two 96 well plates with each RNA sample represented by a single cDNA. Each plate contained a reference cDNA standard curve, over a 625-fold concentration range, in duplicate. Analysis consisted of calculating the  $\Delta CT$  (target gene CT – mean reference cDNA CT).  $\Delta CT$  is directly proportional to the negative log<sub>2</sub> fold change. Log<sub>2</sub> fold changes relative to the median non-malignant log<sub>2</sub> fold change were then calculated (log<sub>2</sub> fold change – median normal log<sub>2</sub> fold change). These fold changes were then clustered into frequency classes and graphed.

### Microarray Analysis of Cancer Marker Genes

RNA from 58 gastric tumors and 58 non-malignant ("normal") gastric tissue samples were labeled with Cy5 and hybridized in duplicate or triplicate with Cy3 labeled reference RNA. After normalization, the change in expression in each of 29,718 genes was then estimated by three measures: (i) fold change: the ratio of the gene's median expression (un-standardized) in the tumor samples divided by the median level in the non-malignant samples. (ii) fold change rank and (iii) the statistical probability that the observed fold changes were significant.

### Selection of Serum Markers for Gastric Malignancy

In certain embodiments, the cancer marker can be found in biological fluids, including serum. Serum markers were selected from the array data based on (i) the presence of a signal sequence characteristic of secreted proteins or cleaved from the outside of the membrane, (ii) the median level of over-expression (fold change) in tumors compared to non-malignant controls, (iii) the median change in expression rank between tumors and non-malignant controls, and (iv) the degree of overlap between the ranges of expression in the tumor and the non-malignant controls.

All 29 GTMs are known to have a signal peptide sequence at the 5' end of their coding sequences. The signal sequence targets the GTM proteins for transport to an extracellular compartment through the plasma membrane (Gunner von Heijne, Journal of Molecular Biology 173:243-251 (1984)). In addition, none of the GTMs have transmembrane sequence motifs that would result in the full-length protein being retained within the plasma membrane. Consequently, all of the GTM markers of this invention are likely to be secreted into the extracellular compartment, and therefore can be in contact with the vasculature, either being taken up by capillaries, or by being transported into the lymphatic system and then into the vasculature. As a result, each of these tumor-derived markers will be present in the blood.

Next, genes were excluded if >50% of the tumor samples showed expression levels within the 95<sup>th</sup> percentile of the non-malignant range. The variation in the degree of over-expression in the tumor samples reflects not only tumor heterogeneity but also variations in the extent of contamination of the tumor samples with "normal" tissue including muscle, stromal cells and non-malignant epithelial glands. This "normal" contamination ranged from 5 to 70% with a median of approximately 25%. Other genes were excluded because of high relative expression in hematopoietic cells,

or elevated expression in metaplastic gastric tissue. It can be appreciated that depending on the degree of contamination by normal cells or cells that normally express the marker, different threshold ranges can be selected that can provide sufficient separation between a cancer source and a normal source.

GTM that we have found to be useful include genes (DNA), complementary DNA (cDNA), RNA, proteins, and protein fragments of the following markers: carboxypeptidase N, polypeptide 2, 83 kDa chain (also known as carboxypeptidase N (CPN2), matrix metalloproteinase 12 (MMP12), inhibin ("TNHBA"), insulin-like growth factor 7 ("IGFBP7"), gamma-glutamyl hydrolase ("GGH"), leucine proline-enriched proteoglycan ("LEPRE1"), cystatin S ("CST4"), secreted frizzled-related protein 4 ("SFRP4"), asporin ("ASPN"), cell growth regulator with EF hand domain 1 ("CGREF1"), kallikrein (KLK10), tissue inhibitor of metalloproteinase 1 ("TIMP1"), secreted acidic cysteine-rich protein ("SPARC"), transforming growth factor,  $\beta$ -induced ("TGFB1"), EGF-containing fibulin-like extracellular matrix protein 2 ("EFEMP2"), lumican ("LUM"), stannin ("SNN"), secreted phosphoprotein 1 ("SPP1"), chondroitin sulfate proteoglycan 2 ("CSPG2"), N-acylsphingosine amidohydrolase ("ASAHI"), serine protease 11 ("PRSS11"), secreted frizzled-related protein 2 ("SFRP2"), phospholipase A2, group XIIB ("PLA2G12B"), spondin 2, extracellular matrix protein ("SPON2"), olfactomedin 1 ("OLFM1"), thrombospondin repeat containing 1 ("TSRC1"), thrombospondin 2 ("THBS2"), adlcan, cystatin SA ("CST2"), cystatin SN (CST1), lysyl oxidase-like enzyme 2 ("LOXL2"), thyroglobulin ("TG"), transforming growth factor beta1 ("TGFB1"), serine or cysteine proteinase inhibitor clade H ("SERPINH1"), serine or cysteine proteinase inhibitor clade B ("SERPINB5"), matrix metalloproteinase 2 ("MMP2"), proprotein convertase subtilisin/kexin type 5 ("PCSK5"), and hyaluronan proteoglycan link protein 4 ("HAPLN4").

DNA sequences of GTM of this invention along with identifying information are shown herein below.

#### **Matrix Metalloproteinase 12**

>gi|4505206|ref|NM\_002426.1| Homo sapiens matrix metalloproteinase 12 (macrophage elastase) (MMP12), mRNA | qPCR forward\_primer match [758..780] | qPCR reverse\_primer match [888..864] | qPCR probe match [786..815]

TAGAAGTTTACAATGAAGTTTCTTCTAATACTGCTCCTGCAGGCCA  
 CTGCTTCTGGAGCTCTTCCCCTGAACAGCTCTACAAGCCTGGAAAAAAT  
 AATGTGCTATTTGGTGAGAGATACTTAGAAAAATTTTATGGCCTTGAGATA  
 AACAACTTCCAGTGACAAAAATGAAATATAGTGGAACCTTAATGAAGG  
 AAAAAATCCAAGAAATGCAGCACTTCTTGGGTCTGAAAGTGACCGGGCAA  
 CTGGACACATCTACCCTGGAGATGATGCACGCACCTCGATGTGGAGTCCC  
 CGATCTCCATCATTTTCAGGGAAATGCCAGGGGGGGCCCGTATGGAGGAAAC  
 ATTATATCACCTACAGAATCAATAATTACACACCTGACATGAACCGTGAG  
 GATGTTGACTACGCAATCCGGAAAGCTTTCCAAGTATGGAGTAATGTTAC  
 CCCCTTGAAATTCAGCAAGATTAACACAGGCATGGCTGACATTTTGGTGG  
 TTTTGGCCCGTGGAGCTCATGGAGACTTCCATGCTTTTGATGGCAAAGGTG  
 GAATCCTAGCCCATGCTTTTGGACCTGGATCTGGCATTGGAGGGGATGCA  
 CATTTTCGATGAGGACGAATTCTGGACTACACATTCAGGAGGCACAACTT  
 GTTCCTCACTGCTGTTACGAGATTGGCCATTCTTAGGTCTTGGCCATTCT  
 AGTGATCCAAAGGCTGTAATGTTCCCCACCTACAAATATGTCGACATCAA  
 CACATTTTCGCTCTCTGCTGATGACATACGTGGCATTTCAGTCCCTGTATGG  
 AGACCCAAAAGAGAACCAACGCTTGCCAAATCCTGACAATTCAGAACCCAG  
 CTCTCTGTGACCCCAATTTGAGTTTTGATGCTGTCACTACCGTGGGAAATA  
 AGATCTTTTTCTTCAAAGACAGGTTCTTCTGGCTGAAGGTTTCTGAGAGAC  
 CAAAGACCAGTGTTAATTTAATTTCTTCTTATGGCCAACCTTGCCATCTG  
 GCATTGAAGCTGCTTATGAAATTGAAGCCAGAAATCAAGTTTTCTTTTTA  
 AAGATGACAAATACTGGTTAATTAGCAATTTAAGACCAGAGCCAAATTAT  
 CCAAGAGCATACTTCTTTTGGTTTTCTTAACCTTTGTGAAAAAATTGAT  
 GCAGCTGTTTTTAACCCACGTTTTTATAGGACCTACTTCTTTGTAGATAAC  
 CAGTATTGGAGGTATGATGAAAGGAGACAGATGATGGACCCTGGTTATCC  
 CAACTGATTACCAAGAACTTCCAAGGAATCGGGCCTAAAATTGATGCAG  
 TCTTCTATTCTAAAAACAAATACTACTATTTCTTCCAAGGATCTAACCAAT  
 TTGAATATGACTTCTACTCCAACGTATCACCAAAACACTGAAAAGCAAT  
 AGCTGGTTTGGTTGTTAGAAATGGTGTAATTAATGGTTTTTGTAGTTTAC  
 TTCAGCTTAATAAGTATTTATTGCATATTTGCTATGTCCTCAGTGTACCACT  
 ACTTAGAGATATGTATCATAAAAAATAAAATCTGTAAACCATAGGTAATGA  
 TTATATAAAATACATAATTTTTTCAATTTTGAAAACCTCTAATTGTCCATTC  
 TTGCTTGACTCTACTATTAAGTTTGAAAATAGTTACCTTCAAAGCAAGATA  
 ATTCTATTTGAAGCATGCTCTGTAAGTTGCTTCTAACATCCTTGGAAGTA  
 GAAATTATACTTACTTCTGGCATAACTAAAATTAAGTATATATATTTTGGC  
 TCAAATAAAATTG

SEQ ID NO:67

### Inhibin Beta A

>gi|4504698|ref|NM\_002192.1| Homo sapiens inhibin, beta A (activin A,  
 activin AB alpha polypeptide) (INHBA), mRNA | qPCR assay\_on\_demand\_context  
 match [457..481]

TCCACACACACAAAAACCTGCGCGTGAGGGGGGAGGAAAAGCAG  
 GGCCTTTAAAAAGGCAATCACAACAACCTTTGCTGCCAGGATGCCCTTGCT  
 TTGGCTGAGAGGATTTCTGTTGGCAAGTTGCTGGATTATAGTGAGGAGTTC  
 CCCCACCCAGGATCCGAGGGGGCACAGCGCGGCCCCGACTGTCCGTCTT  
 GTGCGCTGGCCGCCCTCCCAAAGGATGTACCCAACCTCTCAGCCAGAGATG  
 GTGGAGGCCGTCAAGAAGCACATTTTAAACATGCTGCACTTGAAGAAGAG  
 ACCCGATGTCACCCAGCCGGTACCCAAGGCGGCGCTTCTGAACGCGATCA

GAAAGCTTCATGTGGGCAAAGTCGGGGAGAACGGGTATGTGGAGATAGA  
GGATGACATTGGAAGGAGGGCAGAAATGAATGAACTTATGGAGCAGACC  
TCGGAGATCATCACGTTTGCCGAGTCAGGAACAGCCAGGAAGACGCTGCA  
CTTCGAGATTTCCAAGGAAGGCAGTGACCTGTCACTGGTGGAGCGTGCA  
AAGTCTGGCTCTTCCTAAAAGTCCCCAAGGCCAACAGGACCAGGACCAAA  
GTCACCATCCGCCTCTTCCAGCAGCAGAAGCACCCGCAGGGCAGCTTGGA  
CACAGGGGAAGAGGCCGAGGAAGTGGGCTTAAAGGGGGAGAGGAGTGA  
ACTGTTGCTCTCTGAAAAAGTAGTAGACGCTCGGAAGAGCACCTGGCATG  
TCTTCCCTGTCTCCAGCAGCATCCAGCGTTGCTGGACCAGGGCAAGAGC  
TCCCTGGACGTTCCGATTGCCTGTGAGCAGTGCCAGGAGAGTGGCGCCAG  
CTTGGTTCTCCTGGGCAAGAAGAAGAAGAAAGAAGAGGAGGGGGAAGGG  
AAAAAGAAGGGCGGAGGTGAAGGTGGGGCAGGAGCAGATGAGGAAAAG  
GAGCAGTCGCACAGACCTTTCCTCATGCTGCAGGCCCGGCAGTCTGAAGA  
CCACCCTCATCGCCGGCGTCGGCGGGGCTTGGAGTGTGATGGCAAGGTCA  
ACATCTGCTGTAAGAAACAGTTCTTTGTCACTTTCAAGGACATCGGCTGGA  
ATGACTGGATCATTGCTCCCTCTGGCTATCATGCCAACTACTGCGAGGGTG  
AGTGCCCGAGCCATATAGCAGGCACGTCCGGGTCCTCACTGTCCTTCCACT  
CAACAGTCATCAACCACTACCGCATGCGGGGCCATAGCCCCTTTGCCAAC  
CTCAAATCGTGCTGTGTGCCACCAAGCTGAGACCCATGTCCATGTTGTAC  
TATGATGATGGTCAAAACATCATCAAAAAGGACATTCAGAACATGATCGT  
GGAGGAGTGTGGGTGCTCATAGAGTTGCCAGCCAGGGGGAAAGGGAG  
CAAGAGTTGTCCAGAGAAGACAGTGGCAAAATGAAGAAATTTTAAAGGTT  
TCTGAGTTAACCAGAAAAATAGAAATTAAAAACAAAACAAAAACAAAAA  
AAAAACAAAAAAACAAAAGTAAATTAAAAACAAACCTGATGAAACAG  
ATGAAACAGATGAAGGAAGATGTGGAAATCTTAGCCTGCCTTAGCCAGGG  
CTCAGAGATGAAGCAGTGAAGAGACAGATTGGGAGGGGAAAGGGAGAATG  
GTGTACCCTTTATTTCTTCTGAAATCACACTGATGACATCAGTTGTTTAA  
CGGGGTATTGTCCTTTCCCCCTTGAGGTTCCCTTGTGAGCTTGAATCAAC  
CAATCTGATCTGCAGTAGTGTGGACTAGAACAAACCCAAATAGCATCTAGA  
AAGCCATGAGTTTGAAAGGGCCCATCACAGGCACCTTTCCTAGCCTAAT

SEQ ID NO:68

**Insulin-Like Growth Factor Binding Protein 7**

>gi|4504618|ref|NM\_001553.1| Homo sapiens insulin-like growth factor binding protein 7 (IGFBP7), mRNA | qPCR forward\_primer match [470..487] | qPCR reverse\_primer match [567..546] | qPCR probe match [492..517]

GCCGCTGCCACCGCACCCCGCCATGGAGCGGCCGTCGCTGCGCGCC  
CTGCTCCTCGGCGCCGCTGGGCTGCTGCTCCTGCTCCTGCCCTCTCCTCTT  
CCTCCTCTTCGGACACCTGCGGCCCTGCGAGCCGGCCTCCTGCCCGCCCC  
TGCCCCCGCTGGGCTGCCTGCTGGGCGAGACCCGCGACGCGTGCGGCTGC  
TGCCCTATGTGCGCCCGCGGCGAGGGCGAGCCGTGCGGGGGTGGCGGCGC  
CGGCAGGGGGTACTGCGCGCCGGGCATGGAGTGCCTGAAGAGCCGCAAG  
AGGCGGAAGGGTAAAGCCGGGGCAGCAGCCGGCGGTCCGGGTGTAAGCG  
GCGTGTGCGTGTGCAAGAGCCGCTACCCGGTGTGCGGCAGCGACGGCACC  
ACCTACCCGAGCGGCTGCCAGCTGCGCGCCGCCAGCCAGAGGGCCGAGA  
GCCGCGGGGAGAAGGCCATCACCCAGGTCAGCAAGGGCACCTGCGAGCA  
AGGTCCTTCCATAGTGACGCCCCCAAGGACATCTGGAATGTCAGTGGTG  
CCCAGGTGTACTTGAGCTGTGAGGTCATCGGAATCCCGACACCTGTCCTCA

TCTGGAACAAGGTAAAAAGGGGTCACTATGGAGTTCAAAGGACAGAACT  
CCTGCCTGGTGACCGGGACAACCTGGCCATTGAGACCCGGGGTGGCCAG  
AAAAGCATGAAGTAACTGGCTGGGTGCTGGTATCTCCTCTAAGTAAGGAA  
GATGCTGGAGAATATGAGTGCCATGCATCCAATTCCCAAGGACAGGCTTC  
AGCATCAGCAAAAATTACAGTGGTTGATGCCTTACATGAAATACCAGTGA  
AAAAAGGTGAAGGTGCCGAGCTATAAACCTCCAGAATATTATTAGTCTGC  
ATGGTTAAAAAGTAGTCATGGATAACTACATTACCTGTTCTTGCCTAATAAG  
TTTCTTTTAATCCAATCCACTAACACTTTAGTTATATTCACTGGTTTTACAC  
AGAGAAATACAAAATAAAGATCACACATCAAGACTATCTACAAAAATTTA  
TTATATATTTACAGAAGAAAAGCATGCATATCATTAAACAAATAAAATAC  
TTTTTATCACAAAAA

SEQ ID NO: 69

### Gamma-Glutamyl Hydrolase

>gi|4503986|ref|NM\_003878.1| Homo sapiens gamma-glutamyl hydrolase  
(conjugase, folylpolygammaglutamyl hydrolase) (GGH), mRNA | qPCR  
forward\_primer match [531..547] | qPCR reverse\_primer match [611..587] | qPCR  
probe match [549..577]

TGCCGCAGCCCCGCCCGCCCGCAGAGCTTTTGAAAGGCGGCGGG  
AGGCGGCGAGCGCCATGGCCAGTCCGGGCTGCCTGCTGTGCGTGCTGGGC  
CTGCTACTCTGCGGGGCGGCGAGCCTCGAGCTGTCTAGACCCACGGCGA  
CACCGCCAAGAAGCCCATCATCGGAATATTAATGCAAAAATGCCGTAATA  
AAGTCATGAAAAACTATGGAAGATACTATATTGCTGCGTCCTATGTAAAG  
TACTTGGAGTCTGCAGGTGCGAGAGTTGTACCAGTAAGGCTGGATCTTAC  
AGAGAAAGACTATGAAATACTTTTCAAATCTATTAATGGAATCCTTTTCCC  
TGGAGGAAGTGTTGACCTCAGACGCTCAGATTATGCTAAAGTGGCCAAAA  
TATTTTATAACTTGTCCATACAGAGTTTGTATGATGGAGACTATTTTCCTGT  
GTGGGGCACATGCCTTGGATTTGAAGAGCTTTCCTGCTGATTAGTGGAG  
AGTGCTTATTAAGTCCACAGATACTGTTGACGTGGCAATGCCGCTGAACT  
TCACTGGAGGTCAATTGCACAGCAGAATGTTCCAGAATTTTCTACTGAGT  
TGTTGCTGTCAATTAGCAGTAGAACCTCTGACTGCCAATTTCCATAAGTGGA  
GCCTCTCCGTGAAGAATTTTACAATGAATGAAAAGTTAAAGAAGTTTTTC  
AATGTCTTAAGTACAAATACAGATGGCAAGATTGAGTTTATTTCAACAAT  
GGAAGGATATAAGTATCCAGTATATGGTGTCCAGTGGCATCCAGAGAAAG  
CACCTTATGAGTGGAAGAATTTGGATGGCATTTCCTATGCACCTAATGCTG  
TGAAAACCGCATTTTATTTAGCAGAGTTTTTTGTTAATGAAGCTCGGAAAA  
ACAACCATCATTTTAAATCTGAATCTGAAGAGGAGAAAGCATTGATTTAT  
CAGTTCAGTCCAATTTATACTGGAAATATTTCTTCATTTACAGCAATGTTAC  
ATATTTGATTGAAAGTCTTCAATTTGTAAACAGAGCAAATTTGAATAATTC  
CATGATTAACTGTTAGAATAACTTGCTACTCATGGCAAGATTAGGAAGT  
CACAGATTCTTTCTATAATGTGCTGGCTCTGATTCTTCATTATGTATGTG  
ACTATTTATATAACATTAGATAATTAATAGTGAGACATAAATAGAGTGC  
TTTTTCATGGAAAAGCCTTCTTATATCTGAAGATTGAAAAATAAATTTACT  
GAAATACAAAAA

SEQ ID NO: 70



**Leucine Proline-Enriched Proteoglycan 1**

>gi|21361917|ref|NM\_022356.2| Homo sapiens leucine proline-enriched proteoglycan (leprecan) 1 (LEPRE1), mRNA | qPCR forward\_primer match [813..836] | qPCR reverse\_primer match [894..872] | qPCR probe match [841..870]

GGTGGCGGGTGGCTGGCGGTTCCGTTAGGTCTGAGGGAGCGATGG  
CGGTACGCGCGTTGAAGCTGCTGACCACACTGCTGGCTGTCGTGGCCGCT  
GCCTCCCAAGCCGAGGTCGAGTCCGAGGCAGGATGGGGCATGGTGACGCC  
TGATCTGCTCTTCGCCGAGGGGACCGCAGCCTACGCGCGCGGGGACTGGC  
CCGGGGTGGTCCTGAGCATGGAACGGGCGCTGCGCTCCCGGGCAGCCCTC  
CGCGCCCTTCGCCTGCGCTGCCGCACCCAGTGTGCCGCCGACTTCCCGTGG  
GAGCTGGACCCCGACTGGTCCCCCAGCCCGGCCAGGCCTCGGGCGCCGC  
CGCCCTGCGCGACCTGAGCTTCTTCGGGGGCCTTCTGCGTCGCGCTGCCTG  
CCTGCGCCGCTGCCTCGGGCCGCCGGCCGCCACTCGCTCAGCGAAGAGA  
TGGAGCTGGAGTTCCGCAAGCGGAGCCCCTACAACCTACCTGCAGGTGCC  
TACTTCAAGATCAACAAGTTGGAGAAAGCTGTTGCTGCAGCACACACCTT  
CTTCGTGGGCAATCCTGAGCACATGGAAATGCAGCAGAACCTAGACTATT  
ACCAAACCATGTCTGGAGTGAAGGAGGCCGACTTCAAGGATCTTGAGACT  
CAACCCCATATGCAAGAATTCGACTGGGAGTGCGACTCTACTCAGAGGA  
ACAGCCACAGGAAGCTGTGCCCCACCTAGAGGCGGCGCTGCAAGAATACT  
TTGTGGCCTATGAGGAGTGCCGTGCCCTCTGCGAAGGGCCCTATGACTAC  
GATGGCTACAACCTACCTTGAGTACAACGCTGACCTCTTCCAGGCCATCAC  
AGATCATTACATCCAGGTCCTCAACTGTAAGCAGAACTGTGTACGGAGC  
TTGCTTCCACCCAAGTCGAGAGAAAGCCCTTTGAAGACTTCCTCCCATCGC  
ATTATAATTATCTGCAGTTTGCCTACTATAACATTGGGAATTATACACAGG  
CTGTTGAATGTGCCAAGACCTATCTTCTCTTCTTCCCAATGACGAGGTGA  
TGAACCAAAATTTGGCCTATTATGCAGCTATGCTTGGAGAAGAACACACC  
AGATCCATCGGCCCCCGTGAGAGTGCCAAGGAGTACCGACAGCGAAGCCT  
ACTGGA AAAAAGAACTGCTTTTCTTCGCTTATGATGTTTTTGGAAATTCCTTT  
GTGGATCCGGATTCATGGACTCCAGGAGAAGTGATTCCCAAGAGATTGCA  
AGAGAAACAGAAGTCAGAACGGGAAACAGCCGTACGCATCTCCAGGAG  
ATTGGGAACCTTATGAAGGAAATCGAGACCCTTGTGGAAGAGAAGACCA  
AGGAGTCACTGGATGTGAGCAGACTGACCCGGGAAGGTGGCCCCCTGCTG  
TATGAAGGCATCAGTCTCACCATGAACTCCAACTCCTGAATGGTTCCCA  
GCGGGTGGTGATGGACGGCGTAATCTCTGACCACGAGTGTGAGGAGCTGC  
AGAGACTGACCAATGTGGCAGCAACCTCAGGAGATGGCTACCGGGGTCA  
GACCTCCCCACATACTCCCAATGAAAAGTTCTATGGTGTCAGTGTCTTCAA  
AGCCCTCAAGCTGGGGCAAGAAGGCAAAGTTCTCTGCAGAGTGCCACC  
TGTA TACAACGTGACGGAGAAGGTGCGGCGCATCATGGAGTCCTACTTC  
CGCCTGGATACGCCCTCTACTTTTCTCTACTCTCATCTGGTGTGCCGCACT  
GCCATCGAAGAGGTCCAGGCAGAGAGGAAGGATGATAGTCATCCAGTCC  
ACGTGGACAACCTGCATCCTGAATGCCGAGACCCTCGTGTGTGTCAAAGAG  
CCCCAGCCTACACCTTCCGCGACTACAGCGCCATCCTTTACCTAAATGGG  
GACTTCGATGGCGGAACTTTTATTTCACTGAACTGGATGCCAAGACCGT  
GACGGCAGAGGTGCAGCCTCAGTGTGGAAGAGCCGTGGGATTCTCTTCAG  
GCACTGAAAACCCACATGGAGTGAAGGCTGTCACCAGGGGGCAGCGCTGT  
GCCATCGCCCTGTGGTTCACCCTGGACCCTCGACACAGCGAGCGGGTGAG  
AGCAGCTCGAGCGGGTGAGAGCAGCTGGTGCTGTGGTGACCCGTTCCAG

AGCGCCCTTGGTTTGCCTTTCTCTTCCCCAAATCCCATTGCCAGTGGCTGA  
GACACGAAAGGAGCACTTGGGACACCAGCTCCAACGCCCTGTCAATTATGG  
TCACATTGCCTTGTCTCCCTGGGCCTGCTGTGAACGGGATCCAGGTGGGG  
AAAGAGGTCAAGACAGGGAGCGATGCTGAGTTCTTGGTTCCTCCTTGGG  
CCCCACTTCAGCTGTCTTTTCCAGAGAGTAGGACCTGCTGGGAAGGAGA  
TGAGCCTGGGGCCATTAAGGAACCTTCCTTGTCCCCTGGGAAGTAGCAGC  
TGAGAGATAGCGAGTGTCTGGAGCGGAGGCCTCTCTGAATGGGCAGGGGT  
TTGTCTTGCAGGACAGGGTGCAGGCAGATGACCTGGTGAAGATGCTCTT  
CAGCCCAGAAGAGATGGTCCTCTCCCAGGAGCAGCCCCTGGATGCCCAGC  
AGGGCCCCCCCCGAACCTGCACAAGAGTCTCTCTCAGGCAGTGAATCGAAG  
CCCAAGGATGAGCTATGACAGCGTCCAGGTCAGACGGATGGGTGACTAGA  
CCCATGGAGAGGAACTCTTCTGCACTCTGAGCTGGCCAGCCCCCTCGGGGC  
TGCAGAGCAGTGAGCCTACATCTGCCACTCAGCCGAGGGGACCCTGCTCA  
CAGCCTTCTACATGGTGCTACTGCTCTTGGAGTGGACATGACCAGACACC  
GCACCCCCTGGATCTGGCTGAGGGCTCAGGACACAGGCCCAGCCACCCCC  
AGGGGCCTCCACAGGCCGCTGCATAACAGCGATACAGTACTTAAGTGTCT  
GTGTAGACAACCAAAGAATAAATGATTCATGGTTTTTTTT

SEQ ID NO: 71

**Cystatin S**

>gi|19882254|ref|NM\_001899.2| Homo sapiens cystatin S (CST4), mRNA |  
qPCR forward\_primer match [343..361] | qPCR reverse\_primer match [434..411] |  
qPCR probe match [382..410]

GGCTCTCACCTCCTCTCCTGCAGCTCCAGCTTTGTGCTCTGCCTCT  
GAGGAGACCATGGCCCGGCCTCTGTGTACCCTGCTACTCCTGATGGCTACC  
CTGGCTGGGGCTCTGGCCTCGAGCTCCAAGGAGGAGAATAGGATAATCCC  
AGGTGGCATCTATGATGCAGACCTCAATGATGAGTGGGTACAGCGTGCCC  
TTCACCTCGCCATCAGCGAGTACAACAAGGCCACCGAAGATGAGTACTAC  
AGACGCCCGCTGCAGGTGCTGCGAGCCAGGGAGCAGACCTTTGGGGGGGT  
GAATTACTTCTTCGACGTAGAGGTGGGCCGCACCATATGTACCAAGTCCC  
AGCCCAACTTGGACACCTGTGCCTTCCATGAACAGCCAGAACTGCAGAAG  
AAACAGTTGTGCTCTTTCGAGATCTACGAAGTTCCCTGGGAGGACAGAAT  
GTCCCTGGTGAATTCCAGGTGTCAAGAAGCCTAGGGGTCTGTGCCAGGCC  
AGTCACACCGACCACCACCACTCCCACCACTGTAGTGCTCCCACCCCTG  
GACTGGTGGCCCCCACCCTGCGGGAGGCCTCCCCATGTGCCTGTGCCAAG  
AGACAGACAGAGAAGGCTGCAGGAGTCTTTGTTGCTCAGCAGGGCGCTC  
TGCCCTCCCTCCTTCCTTCTTGCTTCTAATAGACCTGGTACATGGTACACAC  
ACCCCCACCTCCTGCAATTAAACAGTAGCATCGCC

SEQ ID NO: 72

**Secreted Frizzles-Related Protein 4**

>gi|8400733|ref|NM\_003014.2| Homo sapiens secreted frizzled-related protein  
4 (SFRP4), mRNA | qPCR assay\_on\_demand\_context match [1079..1103]

GGCGGGTTCGCGCCCCGAAGGCTGAGAGCTGGCGCTGCTCGTGCCC  
TGTGTGCCAGACGGCGGAGCTCCGCGGCCGGACCCCGCGGCCCGCTTTG  
CTGCCGACTGGAGTTTGGGGGAAGAACTCTCCTGCGCCCCAGAAGATTT  
CTTCCTCGGCGAAGGGACAGCGAAAGATGAGGGTGGCAGGAAGAGAAGG  
CGCTTCTGTCTGCCGGGGTGCAGCGCGAGAGGGCAGTGCCATGTTCTCT

TCCATCCTAGTGGCGCTGTGCCTGTGGCTGCACCTGGCGCTGGGCGTGCGC  
GGCGCGCCCTGCGAGGCGGTGCGCATCCCTATGTGCCGGCACATGCCCTG  
GAACATCACGCGGATGCCCAACCACCTGCACCACAGCACGCAGGAGAAC  
GCCATCCTGGCCATCGAGCAGTACGAGGAGCTGGTGGACGTGAACTGCAG  
CGCCGTGCTGCGCTTCTTCTTCTGTGCCATGTACGCGCCCATTTGCACCCT  
GGAGTTCCTGCACGACCCTATCAAGCCGTGCAAGTCGGTGTGCCAACGCG  
CGCGCGACGACTGCGAGCCCCTCATGAAGATGTACAACCACAGCTGGCCC  
GAAAGCCTGGCCTGCGACGAGCTGCCTGTCTATGACCGTGGCGTGTGCAT  
TTCGCCTGAAGCCATCGTCACGGACCTCCCGGAGGATGTTAAGTGGATAG  
ACATCACACCAGACATGATGGTACAGGAAAGGCCTCTTGATGTTGACTGT  
AAACGCCTAAGCCCCGATCGGTGCAAGTGTA AAAAGGTGAAGCCAAC TTT  
GGCAACGTATCTCAGCAAAA ACTACAGCTATGTTATTCATGCCAAAATAA  
AAGCTGTGCAGAGGAGTGGCTGCAATGAGGTCACAACGGTGGTGGATGTA  
AAAGAGATCTTCAAGTCCTCATCACCCATCCCTCGAACTCAAGTCCCGCTC  
ATTACAAATTCTTCTTGCCAGTGTCCACACATCCTGCCCCATCAAGATGTT  
CTCATCATGTGTTACGAGTGGCGTTCAAGGATGATGCTTCTTGAAAATTGC  
TTAGTTGAAAAATGGAGAGATCAGCTTAGTAAAAGATCCATACAGTGGGA  
AGAGAGGCTGCAGGAACAGCGGAGAACAGTTTCAGGACAAGAAGAAAACA  
GCCGGGCGCACCAAGTCGTAGTAATCCCCCAAACCAAAGGGAAAGCCTCC  
TGCTCCCAAACCAAGCCAGTCCCAAGAAGAACATTAAAACTAGGAGTGCCC  
AGAAGAGAACAAACCCGAAAAGAGTGTGAGCTAACTAGTTTCCAAAGCG  
GAGACTTCGACTTCCTTACAGGATGAGGCTGGGCATTGCCTGGGACAGC  
CTATGTAAGGCCATGTGCCCTTGCCCTAACAACTCACTGCAGTGCTCTTC  
ATAGACACATCTTGCAGCATTTTTCTTAAGGCTATGCTTCAGTTTTTCTTTG  
TAAGCCATCACAAAGCCATAGTGGTAGGTTTGCCCTTTGGTACAGAAGGTG  
AGTTAAAGCTGGTGGAAAAGGCTTATTGCATTGCATTACAGAGTAACCTGT  
GTGCATACTCTAGAAGAGTAGGGAAAATAATGCTTGTTACAATTCGACCT  
AATATGTGCATTGTAAAATAAATGCCATATTTCAAACAAAACACGTAATT  
TTTTTACAGTATGTTTTATTACCTTTTGATATCTGTTGTTGCAATGTTAGTG  
ATGTTTTAAATGTGATGAAAATATAATGTTTTTAAGAAGGAACAGTAGT  
GGAATGAATGTAAAAGATCTTTATGTGTTTATGGTCTGCAGAAGGATTTT  
TGTGATGAAAGGGGATTTTTTGAAAAATTAGAGAAGTAGCATATGGAAAA  
TTATAATGTGTTTTTTTACCAATGACTTCAGTTTCTGTTTTTAGCTAGAAAC  
TAAAAACAAAAATAATAAAGAAAAATAATAAAAAAGGAGAGGCAG  
ACAATGTCTGGATTCTGTTTTTTGTTACCTGATTTCCATGATCATGATGC  
TTCTTGTC AACACCCTCTTAAGCAGCACCAGAAACAGTGAGTTTGTCTGTA  
CCATTAGGAGTTAGGTACTAATTAGTTGGCTAATGCTCAAGTATTTTATAC  
CCACAAGAGAGGTATGTCACTCATCTTACTTCCCAGGACATCCACCCTGA  
GAATAATTTGACAAGCTTAAAAATGGCCTTCATGTGAGTGCCAAATTTTGT  
TTTTCTTCATTTAAATATTTTCTTTGCCTAAATACATGTGAGAGGAGTTAA  
ATATAAATGTACAGAGAGGAAAGTTGAGTTCCACCTCTGAAATGAGAATT  
ACTTGACAGTTGGGATACTTTAATCAGAAAAAAGAACTTATTTGCAGCA  
TTTTATCAACAAATTTTATAATTGTGGACAATTGGAGGCATTTATTTTAAA  
AAACAATTTTATTGGCCTTTTGCTAACACAGTAAGCATGTATTTTATAAGG  
CATTCAATAAATGCACAACGCCCAAAGGAAATAAAATCCTATCTAATCCT  
ACTCTCCACTACACAGAGGTAATCACTATTAGTATTTTGGCATATTATTCT  
CCAGGTGTTTGCTTATGCACTTATAAAATGATTTGAACAAATAAACTAG  
GAACCTGTATACATGTGTTTCATAACCTGCCTCCTTTGCTTGGCCCTTTATT  
GAGATAAGTTTTCTGTCAAGAAAGCAGAAACCATCTCATTTCTAACAGC  
TGTGTTATATTCCATAGTATGCATTACTCAACAACTGTTGTGCTATTGGA

TACTTAGGTGGTTTCTTCACTGACAATACTGAATAAACATCTCACCGGAAT  
TC  
SEQ ID NO: 73

### Asporin

>gi|41350213|ref|NM\_017680.3| Homo sapiens asporin (LRR class 1)  
(ASPN), mRNA | qPCR forward\_primer match [798..823] | qPCR reverse\_primer  
match [934..912] | qPCR probe match [842..875]

AGTACTAACATGGACTAATCTGTGGGAGCAGTTTATTCCAGTATCA  
CCCAGGGTGCAGCCACACCAGGACTGTGTTGAAGGGTGTTTTTTTCTTTT  
AAATGTAATACCTCCTCATCTTTTCTTCTTACACAGTGTCTGAGAACATTT  
ACATTATAGATAAGTAGTACATGGTGGATAACTTCTACTTTTAGGAGGACT  
ACTCTCTTCTGACAGTCCTAGACTGGTCTTCTACACTAAGACACCATGAAG  
GAGTATGTGCTCCTATTATTCCTGGCTTTGTGCTCTGCCAAACCTTCTTTA  
GCCCTTCACACATCGCACTGAAGAATATGATGCTGAAGGATATGGAAGAC  
ACAGATGATGATGATGATGATGATGATGATGATGATGATGATGATGAGGA  
CAACTCTCTTTTCCAACAAGAGAGCCAAGAAGCCATTTTTTCCATTTGA  
TCTGTTTCCAATGTGTCCATTTGGATGTCAGTGCTATTACGAGTTGTACA  
TTGCTCAGATTTAGGTTTGACCTCAGTCCCAACCAACATTCCATTTGATAC  
TCGAATGCTTGATCTTCAAAACAATAAAATTAAGGAAATCAAAGAAAATG  
ATTTTAAAGGACTCACTTCACTTTATGGTCTGATCCTGAACAACAACAAGC  
TAACGAAGATTCACCCAAAAGCCTTTCTAACCACAAAGAAGTTGCGAAGG  
CTGTATCTGTCCCAATCAACTAAGTGAAATACCACTTAATCTTCCCAAA  
TCATTAGCAGAACTCAGAATTCATGAAAATAAAGTTAAGAAAATACAAAA  
GGACACATTCAAAGGAATGAATGCTTTACACGTTTTGGAAATGAGTGCAA  
ACCCTCTTGATAATAATGGGATAGAGCCAGGGGCATTTGAAGGGGTGACG  
GTGTTCCATATCAGAATTGCAGAAGCAAAACTGACCTCAGTTCCTAAAGG  
CTTACCACCAACTTTATTTGGAGCTTCACTTAGATTATAATAAAATTTCAAC  
AGTGGAACCTTGAGGATTTTAAACGATACAAAGAACTACAAAGGCTGGGCC  
TAGGAAACAACAAAATCACAGATATCGAAAATGGGAGTCTTGCTAACATA  
CCACGTGTGAGAGAAATACATTTGGAAAACAATAAACTAAAAAAAATCCC  
TTCAGGATTACCAGAGTTGAAATACCTCCAGATAATCTTCCTTCATTCTAA  
TTCAATTGCAAGAGTGGGAGTAAATGACTTCTGTCCAACAGTGCCAAAGA  
TGAAGAAATCTTTATACAGTGCAATAAGTTTATTCAACAACCCGGTGAAA  
TACTGGGAAATGCAACCTGCAACATTTGTTGTGTTTTGAGCAGAATGAGT  
GTTCACTTGGGAACTTTGGAATGTAATAATTAGTAATTGGTAATGTCCAT  
TTAATATAAGATTCAAAAATCCCTACATTTGGAATACTTGAACCTCTATTAA  
TAATGGTAGTATTATATATACAAGCAAATATCTATTCTCAAGTGGTAAGTC  
CACTGACTTATTTTATGACAAGAAATTTCAACGGAATTTTGCCAAACTATT  
GATACATAAGGGTTGAGAGAAACAAGCATCTATTGCAGTTTCTTTTTGCGT  
ACAAATGATCTTACATAAAATCTCATGCTTGACCATTCTTTCTTCATAACA  
AAAAAGTAAGATATTCGGTATTTAACACTTTGTTATCAAGCATATTTTAAA  
AAGAACTGTACTGTAAATGGAATGCTTGACTTAGCAAAATTTGTGCTCTTT  
CATTTGCTGTTAGAAAAACAGAAATTAACAAAGACAGTAATGTGAAGAGTG  
CATTACACTATTCTTATTCTTTAGTAACTTGGGTAGTACTGTAATATTTTAA  
ATCATCTTAAAGTATGATTTGATATAATCTTATTGAAATTACCTTATCATG  
TCTTAGAGCCCGTCTTTATGTTTAAACTAATTTCTTAAATAAAGCCTTC  
AGTAAATGTTTCAATACCAACTTGATAAATGCTACTCATAAGAGCTGGTTTG  
GGGCTATAGCATATGCTTTTTTTTTTTTAAATTATTACCTGATTTAAAAATCT

CTGTAAAAACGTGTAGTGTTCATAAAATCTGTAACTCGCATTTTAATGAT  
 CCGCTATTATAAGCTTTTAATAGCATGAAAATTGTTAGGCTATATAACATT  
 GCCACTTCAACTCTAAGGAATATTTTTGAGATATCCCTTTGGAAGACCTTG  
 CTTGGAAGAGCCTGGACACTAACAATTCTACACCAAATTGTCTCTTCAAAT  
 ACGTATGGACTGGATAACTCTGAGAAACACATCTAGTATAACTGAATAAG  
 CAGAGCATCAAATTAAACAGACAGAAACCGAAAGCTCTATATAAATGCTC  
 AGAGTTCTTTATGTATTTCTTATTGGCATTCAACATATGTAAAATCAGAAA  
 ACAGGGAAATTTTCATTAAAAATATTGGTTTGAAATAAAAAAAAAAAAAA

SEQ ID NO: 74

### Cell Growth Regulator with EF Hand Domain 1

>gi|33589823|ref|NM\_006569.2| Homo sapiens cell growth regulator with EF  
 hand domain 1 (CGREF1), mRNA | qPCR forward\_primer match [378..394] | qPCR  
 reverse\_primer match [455..431] | qPCR probe match [396..415]

CGCGCAGCCCCCTCCGGCCGCGGGCGCAGCGGGGGCGCTGGTGGAG  
 CTGCGAAGGGCCAGGTCCGGCGGGCGGGCGGGCGGCTGGCACTGGCTCC  
 GGACTCTGCCCCGCCAGGGCGGGCGGCTCCAGCCGGGAGGGCGACGTGGA  
 GCGGCCACGTGGAGCGGCCCCGGGGAGGCTGGCGGCGGGAGGCGAGGCG  
 CGGGCGGCGCAGCAGCCAGGAGCGCCACGGAGCTGGACCCCCAGAGCC  
 GCGCGGCGCCGAGCAGTTCCAGGAAGGATGTTACCTTTGACGATGACAG  
 TGTTAATCCTGCTGCTGCTCCCCACGGGTCAGGCTGCCCCAAAGGATGGA  
 GTCACAAGGCCAGACTCTGAAGTGCAGCATCAGCTCCTGCCAAACCCCTT  
 CCAGCCAGGCCAGGAGCAGCTCGGACTTCTGCAGAGCTACCTAAAGGGAC  
 TAGGAAGGACAGAAGTGCAACTGGAGCATCTGAGCCGGGAGCAGGTTCT  
 CCTCTACCTCTTTGCCCTCCATGACTATGACCAGAGTGGACAGCTGGATGG  
 CCTGGAGCTGCTGTCCATGTTGACAGCTGCTCTGGCCCCTGGAGCTGCCAA  
 CTCTCCTACCACCAACCCGGTGATATTGATAGTGGACAAAGTGCTCGAGA  
 CGCAGGACCTGAATGGGGATGGGCTCATGACCCCTGCTGAGCTCATCAAC  
 TTCCCGGGAGTAGCCCTCAGGCACGTGGAGCCCGGAGAGCCCCTTGCTCC  
 ATCTCCTCAGGAGCCACAAGCTGTTGGAAGGCAGTCCCTATTAGCTAAAA  
 GCCCATTAAGACAAGAAACACAGGAAGCCCCTGGTCCCAGAGAAGAAGC  
 AAAGGGCCAGGTAGAGGCCAGAAGGGAGTCTTTGGATCCTGTCCAGGAG  
 CCTGGGGGCCAGGCAGAGGCTGATGGAGATGTTCCAGGGCCCAGAGGGG  
 AAGCTGAGGGCCAGGCAGAGGCTAAAGGAGATGCCCCCTGGGCCCAGAGG  
 GGAAGCTGGGGGCCAGGCAGAGGCTGAAGGAGATGCCCCCGGGCCCAGA  
 GGGGAAGCTGGGGGCCAGGCAGAGGCCAGGGAGAATGGAGAGGAGGCC  
 AAGGAACTTCCAGGGGAAACACTGGAGTCTAAGAACACCCAAAATGACTT  
 TGAGGTGCACATTGTTCAAGTGGAGAATGATGAGATCTAGATCTTGAAGA  
 TACAGGTACCCACGAAGTCTCAGTGCCAGAACATAAGCCCTGAAGTGGG  
 CAGGGGAAATGTACGCTGGGACAAGGACCATCTCTGTGCCCCCTGTCTGG  
 TCCCAGTAGGTATCAGGTCTTTCTGTGCAGCTCAGGGAGACCCTAAGTTAA  
 GGGGCAGATTACCAATAAAGAACTGAATGAATTCATCCCCCGGGCCACC  
 TCTCTACCCGTCCAGCCTGCCAGACCCTCTCAGAGGAACGGGGTTGGGG  
 ACCGAAAGGACAGGGATGCCGCCTGCCAGTGTTTCTGGGCCTCACGGTG  
 CTCCGGCAGCAGAGCGCATGGTGCTAGCCATGGCCGGCTGCAGAGGACCC  
 AGTGAGGAAAGCTCAGTCTATCCCTGGGCCCCAAACCCCTCACCGGTTCCC  
 CCTCACCTGGTGTTCAGACACCCCATGCTCTCCTGCAGCTCAGGGCAGGTG  
 ACCCCATCCCCAGTAATATTAATCATCACTAGAACTTTTGGAGAGCCTTGT  
 ACACATCAGGCATCATGCTGGGCATTTTATATATGATTTTATCCTCACAAT

AATTCTGTAGCCAAGCAGAATTGGTTCCATTTGACAGATGAAGAAATTGA  
GGCAGATTGCGTTAAGTGCTGTACCCTAAGGTGATATGCAGCTAATTAAA  
TGGCAGATTTGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA  
SEQ ID NO: 75

#### Kallikrein 10, Transcript Variant 1

>gi|22208981|ref|NM\_002776.3| Homo sapiens kallikrein 10 (KLK10),  
transcript variant 1, mRNA | qPCR forward\_primer match [851..874] | qPCR  
reverse\_primer match [950..931] | qPCR probe match [890..914]

CATCCTGCCACCCCTAGCCTTGCTGGGGACGTGAACCCCTCTCCCCG  
CGCCTGGGAAGCCTTCTTGACCCGGGACCCGGAGAATCCCCACGGAAGC  
CAGTTCCAAAAGGGATGAAAAGGGGGCGTTTCGGGCACTGGGAGAAGCC  
TGTATTCCAGGGCCCCCTCCCAGAGCAGGAATCTGGGACCCAGGAGTGCCA  
GCCTCACCCACGCAGATCCTGGCCATGAGAGCTCCGCACCTCCACCTCTCC  
GCCGCTCTGGCGCCCGGGCTCTGGCGAAGCTGCTGCCGCTGCTGATGGC  
GCAACTCTGGGCCGAGAGGGCGGCGCTGCTCCCCAAAACGACACGCGCT  
TGGACCCCGAAGCCTATGGCTCCCCGTGCGCGCGCGGCTCGCAGCCCTGG  
CAGGTCTCGCTCTTCAACGGCCTCTCGTTCCACTGCGCGGGTGTCTGGTG  
GACCAGAGTTGGGTGCTGACGGCCGCGCACTGCGGAAACAAGCCACTGTG  
GGCTCGAGTAGGGGATGACCACCTGCTGCTTCTTCAGGGAGAGCAGCTCC  
GCCGGACCACTCGCTCTGTTGTCCATCCCAAGTACCACCAGGGCTCAGGC  
CCCATCCTGCCAAGGCGAACGGATGAGCACGATCTCATGTTGCTGAAGCT  
GGCCAGGCCCGTAGTGCTGGGGCCCCGCGTCCGGGGCCCTGCAGCTTCCCT  
ACCGCTGTGCTCAGCCCGGAGACCAGTGCCAGGTTGCTGGCTGGGGCACC  
ACGGCCGCCCCGAGAGTGAAGTACAACAAGGGCCTGACCTGCTCCAGCAT  
CACTATCCTGAGCCCTAAAGAGTGTGAGGTCTTCTACCCTGGCGTGGTCAC  
CAACAACATGATATGTGCTGGACTGGACCGGGGCCAGGACCCCTTGCCAGA  
GTGACTCTGGAGGCCCCCTGGTCTGTGACGAGACCCTCCAAGGCATCCTCT  
CGTGGGGTGTTTACCCCTGTGGCTCTGCCCAGCATCCAGCTGTCTACACCC  
AGATCTGCAAATACATGTCCTGGATCAATAAAGTCATACGCTCCAACTGA  
TCCAGATGCTACGCTCCAGCTGATCCAGATGTTATGCTCCTGCTGATCCAG  
ATGCCAGAGGGCTCCATCGTCCATCCTCTTCCCTCCCCAGTCGGCTGAACTC  
TCCCCTTGTCTGCACTGTTCAAACCTCTGCCGCCCTCCACACCTCTAAACA  
TCTCCCCTCTCACCTCATTCACCCACCTATCCCCATTCTCTGCCTGTACTGA  
AGCTGAAATGCAGGAAGTGGTGGCAAAGGTTTATTCCAGAGAAGCCAGG  
AAGCCGGTCATCACCCAGCCTCTGAGAGCAGTTACTGGGGTCACCCAACC  
TGACTTCCTCTGCCACTCCCTGCTGTGTGACTTTGGGCAAGCCAAGTGCCC  
TCTCTGAACCTCAGTTTCCTCATCTGCAAAATGGGAACAATGACGTGCCTA  
CCTCTTAGACATGTTGTGAGGAGACTATGATATAACATGTGTATGTAAATC  
TTCATGGTGATTGTCATGTAAGGCTTAACACAGTGGGTGGTGAGTTCTGAC  
TAAAGGTTACCTGTTGTCTGTA  
SEQ ID NO: 76

#### Kallikrein 10 Transcript Variant 2

>gi|22208983|ref|NM\_145888.1| Homo sapiens kallikrein 10 (KLK10),  
transcript variant 2, mRNA | qPCR forward\_primer match [714..737] | qPCR  
reverse\_primer match [813..794] | qPCR probe match [753..777]

ACCAGCGGCAGACCACAGGCAGGGCAGAGGCACGTCTGGGTCCCC  
 TCCCTCCTTCCTATCGGCGACTCCCAGGATCCTGGCCATGAGAGCTCCGCA  
 CCTCCACCTCTCCGCCGCTCTGGCGCCCGGGCTCTGGCGAAGCTGCTGCC  
 GCTGCTGATGGCGCAACTCTGGGCCGCGAGAGGCGGCGCTGCTCCCCAAA  
 ACGACACGCGCTTGGACCCCGAAGCCTATGGCTCCCCGTGCGCGCGCGGC  
 TCGCAGCCCTGGCAGGTCTCGCTCTTCAACGGCCTCTCGTTCCACTGCGCG  
 GGTGTCCTGGTGGACCAGAGTTGGGTGCTGACGGCCGCGCACTGCGGAAA  
 CAAGCCACTGTGGGCTCGAGTAGGGGATGACCACCTGCTGCTTCTTCAGG  
 GAGAGCAGCTCCGCCGGACCACTCGCTCTGTTGTCCATCCCAAGTACCAC  
 CAGGGCTCAGGCCCCATCCTGCCAAGGCGAACGGATGAGCACGATCTCAT  
 GTTGCTGAAGCTGGCCAGGCCCGTAGTGCTGGGGCCCCGCGTCCGGGGCC  
 TGCAGCTTCCCTACCGCTGTGCTCAGCCCGGAGACCAGTGCCAGGTTGCTG  
 GCTGGGGCACCACGGCCGCCCGGAGAGTGAAGTACAACAAGGGCCTGAC  
 CTGCTCCAGCATCACTATCCTGAGCCCTAAAGAGTGTGAGGTCTTCTACCC  
 TGGCGTGGTCACCAACAACATGATATGTGCTGGACTGGACCGGGGCCAGG  
 ACCCTTGCCAGAGTGACTCTGGAGGCCCCCTGGTCTGTGACGAGACCCTC  
 CAAGGCATCCTCTCGTGGGGTGTTTACCCCTGTGGCTCTGCCAGCATCCA  
 GCTGTCTACACCCAGATCTGCAAATACATGTCCTGGATCAATAAAGTCAT  
 ACGCTCCAAGTATCCAGATGCTACGCTCCAGCTGATCCAGATGTTATGCT  
 CCTGCTGATCCAGATGCCAGAGGCTCCATCGTCCATCCTCTTCTCCCCA  
 GTCGGCTGAACTCTCCCTTGTCTGCACTGTTCAAACCTCTGCCGCCCTCC  
 ACACCTCTAAACATCTCCCTCTCACCTCATTCCCCACCTATCCCCATTCT  
 CTGCCTGTACTGAAGCTGAAATGCAGGAAGTGGTGGCAAAGGTTTATTCC  
 AGAGAAGCCAGGAAGCCGGTCATCACCCAGCCTCTGAGAGCAGTTACTGG  
 GGTCACCCAACCTGACTTCCTCTGCCACTCCCTGCTGTGTGACTTTGGGCA  
 AGCCAAGTGCCCTCTCTGAACCTCAGTTTCCTCATCTGCAAAATGGGAACA  
 ATGACGTGCCTACCTCTTAGACATGTTGTGAGGAGACTATGATATAACAT  
 GTGTATGTAAATCTTCATGGTGATTGTTCATGTAAGGCTTAACACAGTGGGT  
 GGTGAGTTCTGACTAAAGGTTACCTGTTGTCTGTA

SEQ ID NO: 77

### Tissue Inhibitor of Metalloproteinase 1

>gi|4507508|ref|NM\_003254.1| Homo sapiens tissue inhibitor of  
 metalloproteinase 1 (erythroid potentiating activity, collagenase inhibitor) (TIMP1),  
 mRNA | qPCR forward\_primer match [221..241] | qPCR reverse\_primer match  
 [359..340] | qPCR probe match [251..283]

AGGGGCCTTAGCGTGCCGCATCGCCGAGATCCAGCGCCCAGAGAG  
 ACACCAGAGAACCCACCATGGCCCCCTTTGAGCCCCTGGCTTCTGGCATCC  
 TGTTGTTGCTGTGGCTGATAGCCCCCAGCAGGGCCTGCACCTGTGTCCAC  
 CCCACCCACAGACGGCCTTCTGCAATTCCGACCTCGTCATCAGGGCCAAG  
 TTCGTGGGGACACCAGAAGTCAACCAGACCACCTTATACCAGCGTTATGA  
 GATCAAGATGACCAAGATGTATAAAGGGTTCCAAGCCTTAGGGGATGCCG  
 CTGACATCCGGTTCGTCTACACCCCCGCCATGGAGAGTGTCTGCGGATACT  
 TCCACAGGTCCCACAACCGCAGCGAGGAGTTTCTCATTGCTGGAAAAGT  
 CAGGATGGACTCTTGACATCACTACCTGCAGTTTCGTGGCTCCCTGGAAC  
 AGCCTGAGCTTAGCTCAGCGCCGGGGCTTCACCAAGACCTACACTGTTGG  
 CTGTGAGGAATGCACAGTGTTCCTGTTTATCCATCCCCTGCAAACTGCA  
 GAGTGGCACTCATTGCTTGTGGACGGACCAGCTCCTCCAAGGCTCTGAAA



AGGGCTTCCAGTCCCGTCACCTTGCCTGCCTGCCTCGGGAGCCAGGGCTGT  
GCACCTGGCAGTCCCTGCGGTCCAGATAGCCTGAATCCTGCCCGGAGTG  
GAACTGAAGCCTGCACAGTGTCCACCCTGTTCCCACTCCCATCTTTCTTCC  
GGACAATGAAATAAAGAGTTACCACCCAGC SEQ ID NO: 78

**Secreted Protein, Acidic, Cysteine-Rich**

>gi|48675809|ref|NM\_003118.2| Homo sapiens secreted protein, acidic,  
cysteine-rich (osteonectin) (SPARC), mRNA | qPCR forward\_primer match  
[788..810] | qPCR reverse\_primer match [915..898] | qPCR probe match [818..839]

GTTGCCTGTCTCTAAACCCCTCCACATTCCCGCGGTCCTTCAGACTG  
CCCGGAGAGCGCGCTCTGCCTGCCGCTGCCTGCCTGCCACTGAGGGTTCC  
CAGCACCATGAGGGCCTGGATCTTCTTTCTCCTTTGCCTGGCCGGGAGGGC  
CTTGGCAGCCCCTCAGCAAGAAGCCCTGCCTGATGAGACAGAGGTGGTGG  
AAGAACTGTGGCAGAGGTGACTGAGGTATCTGTGGGAGCTAATCCTGTC  
CAGGTGGAAGTAGGAGAATTTGATGATGGTGCAGAGGAAACCGAAGAGG  
AGGTGGTGGCGGAAAATCCCTGCCAGAACCACCACTGCAAACACGGCAA  
GGTGTGCGAGCTGGATGAGAACAACACCCCATGTGCGTGTGCCAGGACC  
CCACCAGCTGCCCAGCCCCATTGGCGAGTTTGAGAAGGTGTGCAGCAAT  
GACAACAAGACCTTCGACTCTTCCTGCCACTTCTTTGCCACAAAGTGCACC  
CTGGAGGGCACCAAGAAGGGCCACAAGCTCCACCTGGACTACATCGGGCC  
TTGCAAATACATCCCCCCTTGCCTGGACTCTGAGCTGACCGAATTCCCCCT  
GCGCATGCGGGACTGGCTCAAGAACGTCCTGGTCACCCTGTATGAGAGGG  
ATGAGGACAACAACCTTCTGACTGAGAAGCAGAAGCTGCGGGTGAAGAA  
GATCCATGAGAATGAGAAGCGCCTGGAGGCAGGAGACCACCCCGTGGAG  
CTGCTGGCCCCGGGACTTCGAGAAGAATAACATGTACATCTTCCCTGTA  
CACTGGCAGTTCGGCCAGCTGGACCAGCACCCCATTGACGGGTACCTCTC  
CCACACCGAGCTGGCTCCACTGCGTGCTCCCCTCATCCCCATGGAGCATTG  
CACCACCCGCTTTTTCGAGACCTGTGACCTGGACAATGACAAGTACATCG  
CCCTGGATGAGTGGGCCGGCTGCTTCGGCATCAAGCAGAAGGATATCGAC  
AAGGATCTTGTGATCTAAATCCACTCCTTCCACAGTACCGGATTCTCTCTT  
TAACCCTCCCCCTTCGTGTTTCCCCCAATGTTTAAAATGTTTGGATGGTTTGT  
TGTTCTGCCTGGAGACAAGGTGCTAACATAGATTTAAGTGAATACATTAA  
CGGTGCTAAAAATGAAAATTCTAACCCAAGACATGACATTCTTAGCTGTA  
ACTTAACTATTAAGGCCTTTTCCACACGCATTAATAGTCCCATTCTCTCT  
GCCATTTGTAGCTTTGCCCATTTGTCTTATTGGCACATGGGTGGACACGGAT  
CTGCTGGGCTCTGCCTTAAACACACATTGCAGCTTCAACTTTTCTCTT  
GTTCTGTTTGAACTAATACTTACCGAGTCAGACTTTGTGTTCAATTCATT  
CAGGGTCTTGGCTGCCTGTGGGCTTCCCCAGGTGGCCTGGAGGTGGGCAA  
AGGGAAGTAACAGACACACGATGTTGTCAAGGATGGTTTTGGGACTAGAG  
GCTCAGTGGTGGGAGAGATCCCTGCAGAACCCACCAACCAGAACGTGGTT  
TGCTGAGGGCTGTAAGTGAAGAAAGATTCTGGGGCTGTGTTATGAAAAT  
ATAGACATTCTCACATAAGCCAGTTCATCACCATTTCTCCTTTACCTTTC  
AGTGCAGTTTCTTTTACATTAGGCTGTTGGTTCAAACCTTTGGGAGCACG  
GACTGTCAGTTCTCTGGGAAGTGGTCAGCGCATCCTGCAGGGCTTCTCCTC  
CTCTGTCTTTTGGAGAACCAGGGCTCTTCTCAGGGGCTCTAGGGACTGCCA  
GGCTGTTTCAGCCAGGAAGGCCAAAATCAAGAGTGAGATGTAGAAAAGTTG  
TAAAATAGAAAAAGTGGAGTTGGTGAATCGGTTGTTCTTTCTCACATTTG  
GATGATTGTCATAAGGTTTTTAGCATGTTCTCCTTTTCTTACCCTCCCCT



TTTTCTTCTATTAATCAAGAGAACTTCAAAGTTAATGGGATGGTCGGAT  
CTCACAGGCTGAGAACTCGTTCACCTCCAAGCATTTCATGAAAAAGCTGC  
TTCTTATTAATCATACAAACTCTCACCATGATGTGAAGAGTTTCACAAATC  
CTTCAAAATAAAAAAGTAATGACTTAGAACTGCCTTCCTGGGTGATTGTC  
ATGTGTCTTAGTCTTAGTCACCTTATTATCCTGACACAAAAACACATGAGC  
ATACATGTCTACACATGACTACACAAATGCAAACCTTTGCAAACACATTA  
TGCTTTTGCACACACACACCTGTACACACACACCGGCATGTTTATACACAG  
GGAGTGTATGGTTCCTGTAAGCACTAAGTTAGCTGTTTTTCATTTAATGACC  
TGTGGTTTAAACCTTTTGATCACTACCACCATTATCAGCACCAGACTGAGC  
AGCTATATCCTTTTATTAATCATGGTCATTTCATTTCATTTCATTACAAA  
ATATTTATGATGTATTTACTCTGCACCAGGTCCCATGCCAAGCACTGGGGA  
CACAGTTATGGCAAAGTAGACAAAGCATTGTTCATTGGAGCTTAGAGT  
CCAGGAGGAATACATTAGATAATGACACAATCAAATATAAATTGCAAGAT  
GTCACAGGTGTGATGAAGGGAGAGTAGGAGAGACCATGAGTATGTGTAA  
CAGGAGGACACAGCATTATTCTAGTGCTGTACTGTTCCGTACGGCAGCCA  
CTACCCACATGTAACCTTTTAAAGATTAAATTAAATTAGTTAACATTCAA  
AACGCAGCTCCCCAATCACACTAGCAACATTTCAGTGCTTGAGAGCCAT  
GCATGATTAGTGGTTACCCTATTGAATAGGTCAGAAGTAGAATCTTTTCAT  
CATCACAGAAAGTTCTATTGGACAGTGCTCTTCTAGATCATCATAAGACTA  
CAGAGCACTTTTCAAAGCTCATGCATGTTTCATCATGTTAGTGTTCGATTTT  
GAGCTGGGGTGGTTGAGACTCCCCTTAGAGATAGAGAAACAGACCCAAGAA  
ATGTGCTCAATTGCAATGGGCCACATACCTAGATCTCCAGATGTCATTTC  
CCTCTCTTATTTTAAAGTTATGTTAAGATTACTAAAACAATAAAAGCTCCTA  
AAAAATCAA

SEQ ID NO: 79

**Transforming Growth Factor, Beta-Induced**

>gi|4507466|ref|NM\_000358.1| Homo sapiens transforming growth factor,  
beta-induced, 68kDa (TGFBI), mRNA | qPCR assay\_on\_demand\_context match  
[170..194]

GCTTGCCCGTCGGTCGCTAGCTCGCTCGGTGCGCGTCGTCCCGCTCC  
ATGGCGCTCTTCGTGCGGCTGCTGGCTCTCGCCCTGGCTCTGGCCCTGGGC  
CCCGCCGCGACCCTGGCGGGTCCCGCCAAGTCGCCCTACCAGCTGGTGCT  
GCAGCACAGCAGGCTCCGGGGCCGCCAGCACGGCCCCAACGTGTGTGCTG  
TGCAGAAGGTTATTGGCACTAATAGGAAGTACTTCACCAACTGCAAGCAG  
TGGTACCAAAGGAAAATCTGTGGCAAATCAACAGTCATCAGCTACGAGTG  
CTGTCCTGGATATGAAAAGGTCCCTGGGGGAGAAGGGCTGTCCAGCAGCCC  
TACCACTCTCAAACCTTTACGAGACCCTGGGAGTCGTTGGATCCACCACCA  
CTCAGCTGTACACGGACCGCACGGAGAAGCTGAGGCCTGAGATGGAGGG  
GCCCGGCAGCTTCACCATCTTCGCCCTAGCAACGAGGCCTGGGCCTCCTT  
GCCAGCTGAAGTGCTGGACTCCCTGGTCAGCAATGTCAACATTGAGCTGC  
TCAATGCCCTCCGCTACCATATGGTGGGCAGGCGAGTCCTGACTGATGAG  
CTGAAACACGGCATGACCCTCACCTCTATGTACCAGAATTCCAACATCCA  
GATCCACCACTATCCTAATGGGATTGTAAGTGTGAAGTGTGCCCGGCTCCT  
GAAAGCCGACCACCATGCAACCAACGGGGTGGTGCACCTCATCGATAAGG  
TCATCTCCACCATCACCAACAACATCCAGCAGATCATTGAGATCGAGGAC  
ACCTTTGAGACCCTTCGGGCTGCTGTGGCTGCATCAGGGCTCAACACGAT  
GCTTGAAGGTAACGGCCAGTACACGCTTTTGGCCCCGACCAATGAGGCCT

TCGAGAAGATCCCTAGTGAGACTTTGAACCGTATCCTGGGCGACCCAGAA  
 GCCCTGAGAGACCTGCTGAACAACCACATCTTGAAGTCAGCTATGTGTGC  
 TGAAGCCATCGTTGCGGGGCTGTCTGTAGAGACCCTGGAGGGCAGCAGAC  
 TGGAGGTGGGCTGCAGCGGGGACATGCTCACTATCAACGGGAAGGCGATC  
 ATCTCCAATAAAGACATCCTAGCCACCAACGGGGTGATCCACTACATTGA  
 TGAGCTACTCATCCAGACTCAGCCAAGACACTATTTGAATTGGCTGCAG  
 AGTCTGATGTGTCCACAGCCATTGACCTTTTCAGACAAGCCGGCCTCGGCA  
 ATCATCTCTCTGGAAGTGAGCGGTTGACCCTCCTGGCTCCCCTGAATTCTG  
 TATTCAAAGATGGAACCCCTCCAATTGATGCCCATACAAGGAATTTGCTTC  
 GGAACCACATAATTAAGACCAGCTGGCCTCTAAGTATCTGTACCATGGA  
 CAGACCCTGGAACTCTGGGCGGGCAAAAACTGAGAGTTTTTGTATTATCG  
 TAATAGCCTCTGCATTGAGAACAGCTGCATCGCGGCCACGACAAGAGGG  
 GGAGGTACGGGACCCTGTTACGATGGACCGGGTGCTGACCCCCCAATG  
 GGGACTGTCATGGATGTCCTGAAGGGAGACAATCGCTTTAGCATGCTGGT  
 AGCTGCCATCCAGTCTGCAGGACTGACGGAGACCCTCAACCGGGAAGGAG  
 TCTACACAGTCTTTGCTCCCAAAATGAAGCCTTCCGAGCCCTGCCACCAA  
 GAGAACGGAGCAGACTCTTGGGAGATGCCAAGGAACCTGCCAACATCCTG  
 AAATACCACATTGGTGATGAAATCCTGGTTAGCGGAGGCATCGGGGCCCT  
 GGTGCGGCTAAAGTCTCTCCAAGGTGACAAGCTGGAAGTCAGCTTGAAAA  
 ACAATGTGGTGAGTGTCAACAAGGAGCCTGTTGCCGAGCCTGACATCATG  
 GCCACAAATGGCGTGGTCCATGTCATCACCATGTTCTGCAGCCTCCAGCC  
 AACAGACCTCAGGAAAGAGGGGATGAACTTGCAGACTCTGCGCTTGAGAT  
 CTTCAAACAAGCATCAGCGTTTTCCAGGGCTTCCAGAGGTCTGTGCGACT  
 AGCCCCTGTCTATCAAAAGTTATTAGAGAGGATGAAGCATTAGCTTGAAG  
 CACTACAGGAGGAATGCACCACGGCAGCTCTCCGCCAATTTCTCTCAGAT  
 TTCCACAGAGACTGTTTGAATGTTTTCAAAACCAAGTATCACACTTTAATG  
 TACATGGGCCGCACCATAATGAGATGTGAGCCTTGTGCATGTGGGGGAGG  
 AGGGAGAGAGATGTACTTTTTAAATCATGTTCCCCCTAAACATGGCTGTTA  
 ACCCACTGCATGCAGAACTTGGATGTCACTGCCTGACATTCACTTCCAGA  
 GAGGACCTATCCCAAATGTGGAATTGACTGCCTATGCCAAGTCCCTGGAA  
 AAGGAGCTTCAGTATTGTGGGGCTCATAAAACATGAATCAAGCAATCCAG  
 CCTCATGGGAAGTCCTGGCACAGTTTTTGTAAAGCCCTTGACAGCTGGA  
 GAAATGGCATCATTATAAGCTATGAGTTGAAATGTTCTGTCAAATGTGTCT  
 CACATCTACACGTGGCTTGGAGGCTTTTATGGGGCCCTGTCCAGGTAGAA  
 AAGAAATGGTATGTAGAGCTTAGATTTCCCTATTGTGACAGAGCCATGGT  
 GTGTTTGTAAATAATAAAACCAAAGAAACATA

SEQ ID NO: 80

### EGF-Containing Fibulin-Like Extracellular Matrix Protein 2

>gi|8393298|ref|NM\_016938.1| Homo sapiens EGF-containing fibulin-like  
 extracellular matrix protein 2 (EFEMP2), mRNA | qPCR assay\_on\_demand\_context  
 match [1248..1272]

CAAGCTTGGCACGAGGGCAGGCATTGCCCCGAGCCAGCCGAGCCGC  
 CAGAGCCGCGGGCCGCGCGGGTGTCGCGGGCCCAACCCAGGATGCTCCC  
 CTGCGCCTCCTGCCTACCCGGGTCTCTACTGCTCTGGGCGCTGCTACTGTT  
 GCTCTTGGGATCAGCTTCTCCTCAGGATTCTGAAGAGCCCGACAGCTACAC  
 GGAATGCACAGATGGCTATGAGTGGGACCCAGACAGCCAGCACTGCCGG  
 GATGTCAACGAGTGTCTGACCATCCCTGAGGCCTGCAAGGGGGAAATGAA  
 GTGCATCAACCACTACGGGGGCTACTTGTGCCTGCCCCGCTCCGCTGCCGT

CATCAACGACCTACACGGCGAGGGACCCCCGCCACCAGTGCCTCCCGCTC  
 AACACCCCAACCCCTGCCCACCAGGCTATGAGCCCGACGATCAGGACAGC  
 TGTGTGGATGTGGACGAGTGTGCCAGGCCCTGCACGACTGTCGCCCCAG  
 CCAGGACTGCCATAACTTGCCTGGCTCCTATCAGTGCACCTGCCCTGATGG  
 TTACCGCAAGATCGGGCCCCGAGTGTGTGGACATAGACGAGTGCCGCTACC  
 GCTACTGCCAGCACCGCTGCGTGAACCTGCCTGGCTCCTTCCGCTGCCAGT  
 GCGAGCCGGGCTTCCAGCTGGGGCCTAACAACCGCTCCTGTGTTGATGTG  
 AACGAGTGTGACATGGGGGCCCCATGCGAGCAGCGCTGCTTCAACTCCTA  
 TGGGACCTTCTGTGTCGCTGCCACCAGGGCTATGAGCTGCATCGGGATG  
 GCTTCTCCTGCAGTGATATTGATGAGTGTAGCTACTCCAGCTACCTCTGTC  
 AGTACCGCTGCGTCAACGAGCCAGGCCGTTTCTCCTGCCACTGCCACAG  
 GGTTACCAGCTGCTGGCCACACGCCTCTGCCAAGACATTGATGAGTGTGA  
 GTCTGGTGCGCACCAAGTGCTCCGAGGCCCAAACCTGTGTCAACTTCCATG  
 GGGGCTACCGCTGCGTGGACACCAACCGCTGCGTGGAGCCCTACATCCAG  
 GTCTCTGAGAACCGCTGTCTCTGCCCGGCCTCCAACCCTCTATGTCGAGAG  
 CAGCCTTCATCCATTGTGCACCGCTACATGACCATCACCTCGGAGCGGAG  
 AGTACCCGCTGACGTGTTCCAGATCCAGGCGACCTCCGTCTACCCCGGTGC  
 CTACAATGCCTTTCAGATCCGTGCTGGAACTCGCAGGGGGGACTTTTACAT  
 TAGGCAAATCAACAACGTCAGCGCCATGCTGGTCCTCGCCCGGCCGGTGA  
 CGGGCCCCCGGGAGTACGTGCTGGACCTGGAGATGGTCACCATGAATTCC  
 CTCATGAGCTACCGGGCCAGCTCTGTACTGAGGCTCACCGTCTTTGTAGGG  
 GCCTACACCTTCTGAGGAGCAGGAGGGAGCCACCCTCCCTGCAGCTACCC  
 TAGCTGAGGAGCCTGTTGTGAGGGGCGAGAATGAGAAAGGCCAGGGGCC  
 CCCATTGACAGGAGCTGGGAGCTCTGCACCACGAGCTTCAGTCACCCCGA  
 GAGGAGAGGAGGTAACGAGGAGGGCGGACTCCAGGCCCGGCCAGAGA  
 TTTGGACTTGGCTGGCTTGCAGGGGTCTTAAGAACTCCACTCTGGACAG  
 CGCCAGGAGGCCCTGGGTTCCATTCTTAAGAACTCTGCCTCAAAGTGTACATT  
 GGATAAGCCCTAGTAGTTCCCTGGGCCTGTTTTTCTATAAAACGAGGCAAC  
 TGG

SEQ ID NO: 81

### Lumican

>gi|21359858|ref|NM\_002345.2| Homo sapiens lumican (LUM), mRNA |  
 qPCR forward\_primer match [61..84] | qPCR reverse\_primer match [182..162] |  
 qPCR probe match [117..152]

GTATCACTCAGAATCTGGCAGCCAGTTCCTGACAGAGTTCAC  
 AGCATATATTGGTGGATTCTTGTCCATAGTGCATCTGCTTTAAGAATTAAC  
 GAAAGCAGTGTCAAGACAGTAAGGATTCAAACCATTTGCCAAAAATGAGT  
 CTAAGTGCATTTACTCTCTTCCCTGGCATTGATTGGTGGTACCAGTGGCCAG  
 TACTATGATTATGATTTTCCCTATCAATTTATGGGCAATCATCACCAAAC  
 TGTGCACCAGAATGTAAGTGCCTGAAAGCTACCCAAGTGCCATGTACTG  
 TGATGAGCTGAAATTGAAAAGTGTACCAATGGTGCCTCCTGGAATCAAGT  
 ATCTTTACCTTAGGAATAACCAGATTGACCATATTGATGAAAAGGCCTTTG  
 AGAATGTAAGTATCTGCAGTGGCTCATTCTAGATCACAACCTTCTAGAA  
 AACTCCAAGATAAAAGGGAGAGTTTTCTCTAAATTGAAACAACTGAAGAA  
 GCTGCATATAAACCAACAACCTGACAGAGTCTGTGGGCCCCACTTCCCA  
 AATCTCTGGAGGATCTGCAGCTTACTCATAACAAGATCACAAGCTGGGC  
 TCTTTTGAAGGATTGGTAAACCTGACCTTCATCCATCTCCAGCACAATCGG  
 CTGAAAGAGGATGCTGTTTCAGCTGCTTTTAAAGGTCTTAAATCACTCGAA

TACCTTGACTTGAGCTTCAATCAGATAGCCAGACTGCCTTCTGGTCTCCCT  
GTCTCTCTTCTAACTCTCTACTTAGACAACAATAAGATCAGCAACATCCCT  
GATGAGTATTTCAAGCGTTTTAATGCATTGCAGTATCTGCGTTTATCTCAC  
AACGAACTGGCTGATAGTGAATACCTGGAAATTCTTTCAATGTGTATCC  
CTGGTTGAGCTGGATCTGTCTATAACAAGCTTAAAAACATACCAACTGTC  
AATGAAAACCTTGAAAACCTTACCTGGAGGTCAATCAACTTGAGAAGTT  
TGACATAAAGAGCTTCTGCAAGATCCTGGGGCCATTATCCTACTCCAAGA  
TCAAGCATTTGCGTTTGGATGGCAATCGCATCTCAGAAACCAGTCTTCCAC  
CGGATATGTATGAATGTCTACGTGTTGCTAACGAAGTCACTCTTAATTAAT  
ATCTGTATCCTGGAACAATATTTTATGGTTATGTTTTTCTGTGTGTAGTTT  
TCATAGTATCCATATTTTATTACTGTTTATTACTTCCATGAATTTTAAAATC  
TGAGGGGAAATGTTTTGTAAACATTTATTTTTTTTAAAGAAAAGATGAAAG  
GCAGGCCTATTTTCATCACAAGAACACACATATACACGAATAGACATCA  
AACTCAATGCTTTATTTGTAAATTTAGTGTTTTTTTTATTTCTACTGTCAAAT  
GATGTGCAAAACCTTTTACTGGTTGCATGGAAATCAGCCAAGTTTATAAT  
CCTTAAATCTTAATGTTCTCAAAGCTTGGAATTAATACATATGGATGTTA  
CTCTCTTGACCAAATTATCTTGATACATTCAAATTTGTCTGGTTAAAAAA  
TAGGTGGTAGATATTGAGGCCAAGAATATTGCAAAATACATGAAGCTTCA  
TGCATTAAAGAAGTATTTTTAGAATAAGAATTTGCATACTTACCTAGTGA  
AACTTTTCTAGAATTATTTTTCACTCTAAGTCATGTATGTTTCTCTTTGATT  
ATTTGCATGTTATGTTTAATAAGCTACTAGCAAAATAAAACATAGCAAAT  
GAAAAAAAAAAAAAAAAAAAAAAAAAAAAA SEQ ID NO: 82

### Stannin

>gi|29893560|ref|NM\_003498.3| Homo sapiens stannin (SNN), mRNA

AGCGGGGCGGACCGGGCGGGCGGAGCCGGGCCCCGCGGGGCTGCT  
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CCTGAGTTCCAGCCTCACTGAGTGGCCACCCCCAAAGTGCTGCCAGCCGA  
GGAAGCCCCCAGCACTGACCATGTCTATTATGGACCACAGCCCCACCACG  
GGCGTGGTCACAGTCATCGTCATCCTCATTGCCATCGCGGCCCTGGGGGCC  
TTGATCCTGGGCTGCTGGTGCTACCTGCGGCTGCAGCGCATCAGCCAGTCA  
GAGGACGAGGAGAGCATCGTGGGGGATGGGGAGACCAAGGAACCCTTCC  
TGCTGGTGCAGTATTCGGCCAAGGGACCGTGCGTGGAGAGAAAGGCCAA  
GCTGATGACTCCCAACGGCCCGGAAGTCCACGGCTGAGCCAGGATGCAAG  
GCTCCTGGTCCTGTTTGCAGCCGGCCAAGAGGCGCTGGGAGGGGGCAAAAC  
CATACGGATGCGCTGCTGTCTGAGAGGAAGGGCTGACACTTGCTGGCATG  
GCCTCTGCGGGCTTCGTCATCGCATGCACTGATGCCCGGGGACCTGGCTGT  
CCTGGGCTTCCCCTCGGCCTCCAGGTGAGGCTGCCCATTCAGGCACTGG  
GCAGGCCTGACCTTGCTGGGGCTCATGGCCCTGTAGCGCTTTTGTACTTG  
AATGTCTAGCTGAGCCTGTTTTTGTATGGAGCTACTACTGTAATGCGTGAAC  
TAACAAACCTGTGAACTGTAAATAGGCCCTGGAAGCACGTGCTTAAGCC  
CTTTTGCTGATTTTAAAAATATCATCTAGCGCACACGGGACTGGTATTCT  
GGCTGTACTAATGACAAGCTGAGTCAAGACCCTGGAGGGTCATAGGCTTG  
TAAAGGCCACGCCACACTCGGCAGGGGTCTCTCATGTGTGTCCATCTGC  
GTGTATGTCAAGGAAGTGAGATGCCAATTTGGGGTCTTGAGGCTGACCAG  
TTGGGGTGCTTGGGTGATCTCTGCTTCATTAGTCATGGGTGGAAGAAAAA  
CCACACCCCCCGCACCCCTCCGTTCTTTCTGCATAGACTCACTTGTTAAAT  
AGCAGTTCTGTTGAGAGTGGAGTTACTGCAGGGAAGCTACCGGACCTGCC  
TGGGAGCCAGTGAAGGGCGAGTCAGGGCACGCGTCCTGGAGGCTGCCAG

CGTCCTTGTAGCAGAGCAGTTTCTTGCCGCTTGGGTCTTCAGCACGCCAAG  
 CCCCCACCAACCCTCCACCCCGAGTGAAGGCTTCGCTGAAATTGCTTTGG  
 TCCTCATAGAGCCTGTGGTGGCTACTTTTGGTCTGAAACCCACTTGGCCCA  
 GGAAAGAGAAAAGGTTGTATGTTTTGTGTTGGTGTTCCTATTTTCTGCAC  
 TGGAGGGGAGGGGACTGTTGAGGTTCTGTCTTTTTTCTTCTTTTCTCTCC  
 CTCTTCACATCACTTGGCTTCCTTTCCTCTCTGATGACCGTCCGCCTATGGG  
 GTTCTGACTTCACTTTCCTCAGCGGGTCTCCAGTCCCCTGACCCAGCTCTA  
 AAGGCACTTAGGACCCAGGGAACATTTCTCACGTGCACATTCCCCTAAGA  
 GCCACCAGACTGCTTCCTGCCAGCCTGTGCTTGCGGCAGGGAGCCGGGGC  
 AGGGCAGAGGTGAACTTGAAGTTCAGGACTTGACTCTCCACAGGTGGTG  
 AGCTGGTGGCTCTCTGGTGAGCTAGTGTCTCCACAGCCTGTCTCCAAGGCC  
 TCCCCTATGTACATTTCACTGAGCTCACTTTGATTTTTTAATCCCACCACAA  
 GCACATACTAATTTTATTTATGATTCAAATGTGACTCGTGCCTGCCCATCC  
 CTGTAATAGATGGAAGGTCAGCCCCGGCTTAACCACAGAGCACTGGCCCT  
 TCATGGCTGAGCTCAGAGCTCTGGCCTCCTGCTCAGACTAAAGGCACCTCC  
 TCTGGCCTCACCCAAGCCTCTTCTAAAAACCATGTTGAATGAATCCACGTT  
 CTGGAACCCCGAGGCGGGAGAAGTAGGGAGCTGTTTCGTTTAAGCAGCATA  
 CACCTAAATTGGGGGTTTAAACATTAAGTAGGAGCTTGGGGTGGAAGAGG  
 GACAGCCGGCTGGGGCCACCTGAGCAGAAGGTGGTAATGAAACACCTCAG  
 CTGGGCTCTTGGGAGACCTTAGGAAGCAGGAGAGGCAACACCTCTGGCTA  
 CTGATGGTGTGGCAAGTTCAGAAGAGGTGGTGGTGGGGTAGGCGTGATGT  
 CAGCAGAAGCCCTGCAGGCTGGGTGGGCAGGACACGTGGTGGGGGCCAC  
 TGAAACCAGGCCTAGGAGGGGAGAACAAAGTTCCAAAGGTGCCGACTGGAA  
 GAAGGGGGTAAAAGTTTGCTTTGGTGAGTGAGAAAAGGCTGGGGCGTGTG  
 ATCCATCCCCTCACGTTTCAGAACTTCCAGGCTTTCTACCTCGACTCTCAC  
 CACAGCCAGCACATACCTAGGCTGTTTTTCTTCCCTCCACACCTGAGGG  
 ACGCAGCAACAGCTAGGATCTGCATTTTCAGGTTCCGAGCCTGACCCCTG  
 GAACTGACCAGCGCTCGATTGTCAGCCTTGGCCTGGGGGTTTTGACCTTGCC  
 AGTGAAGTTTCGGTTTTTGAAGTGATTAAATGTCACTTCCTCATCAGTTTCA  
 CTTCTGGAGGTTTTTCTTATCCTACTCCCTGGTGCCAGGGACGTACCTGGGA  
 GTTTGAATCAGGCCCATTTGAGCGTGGCAGCCGTGTTGGGTGAAGGTCCG  
 GGGCTCGGTGAGGCACTGGGGGGGTTTTTCGGGAGGAAAATGAAAATGCTT  
 CTAGAATGAGTGAACCACATCATAGCTCTCACTGTTTTTTTCAATAGCTACT  
 TTTTTTAGCAGACACCAGAGCCACACTCAAATGGCTAAGTAGGTTATGAC  
 CTCTCTGGATTATTTTTTGAATGCCCAACTGTTGCATTCAAGTTTTCTGACTA  
 ATAAGAAATTAAGCATTTCATCCTTCGTATCACTGCAGAAGCAACAGTGGG  
 GGCACAGGGAGGGAACCTTTGACACTGAGCCACTAAAATATGGACTAATT  
 TTTTGGACAAATCTTCAAACGGACTGTGCTACTGTATTTGTCTCAAAGCTA  
 CCAAGTTTGTGCAATAAGTGGAAGGGATGTCATCCTTCTTCAATAAATGCT  
 GAATGACATTCAAGCTGATTTTCTAGACCACTGAGAAAATCTTTATTTACA  
 ATAAATTTCAATAAAATTTGCATAAATATATCCCAAAAAAAAAAAAAAAAAA  
 AAAAAAGAAAAAAAAAAAAAAAAA

SEQ ID NO: 83

**Secreted Phosphoprotein 1**

>gi|38146097|ref|NM\_000582.2| Homo sapiens secreted phosphoprotein 1  
 (osteopontin, bone sialoprotein I, early T-lymphocyte activation 1) (SPP1), mRNA |  
 qPCR assay\_on\_demand\_context match [253..277]

CTCCCTGTGTTGGTGGAGGATGTCTGCAGCAGCATTAAATTCTGG  
 GAGGGCTTGGTTGTCAGCAGCAGCAGGAGGAGGCAGAGCACAGCATCGT  
 CGGGACCAGACTCGTCTCAGGCCAGTTGCAGCCTTCTCAGCCAAACGCCG  
 ACCAAGGAAAACTCACTACCATGAGAATTGCAGTGATTGCTTTTGCCTCC  
 TAGGCATCACCTGTGCCATACCAGTTAAACAGGCTGATTCTGGAAGTTCTG  
 AGGAAAAGCAGCTTTACAACAAATACCCAGATGCTGTGGCCACATGGCTA  
 AACCCTGACCCATCTCAGAAGCAGAATCTCCTAGCCCCACAGACCCTTCC  
 AAGTAAGTCCAACGAAAGCCATGACCACATGGATGATATGGATGATGAA  
 GATGATGATGACCATGTGGACAGCCAGGACTCCATTGACTCGAACGACTC  
 TGATGATGTAGATGACACTGATGATTCTCACCAGTCTGATGAGTCTCACCA  
 TTCTGATGAATCTGATGAACCTGGTCACTGATTTTCCACGGACCTGCCAGC  
 AACCGAAGTTTTCACTCCAGTTGTCCCCACAGTAGACACATATGATGGCC  
 GAGGTGATAGTGTGGTTTATGGACTGAGGTCAAAATCTAAGAAGTTTCGC  
 AGACCTGACATCCAGTACCCTGATGCTACAGACGAGGACATCACCTCACA  
 CATGGAAAGCGAGGAGTTGAATGGTGCATACAAGGCCATCCCCGTTGCC  
 AGGACCTGAACGCGCCTTCTGATTGGGACAGCCGTGGGAAGGACAGTTAT  
 GAAACGAGTCAGCTGGATGACCAGAGTGCTGAAACCCACAGCCACAAGC  
 AGTCCAGATTATATAAGCGGAAAGCCAATGATGAGAGCAATGAGCATTCC  
 GATGTGATTGATAGTCAGGAACTTTCCAAAGTCAGCCGTGAATTCCACAG  
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 AGGAAGAAGATAAACACCTGAAATTTTCGTATTTCTCATGAATTAGATAGT  
 GCATCTTCTGAGGTCAATTAAAAGGAGAAAAAATACAATTTCTCACTTTG  
 CATTTAGTCAAAAGAAAAAATGCTTTATAGCAAAATGAAAGAGAACATGA  
 AATGCTTCTTTCTCAGTTTATTGGTTGAATGTGTATCTATTTGAGTCTGGAA  
 ATAATAATGTGTTTGATAATTAGTTTGTGGCTTCATGGAAACTCC  
 CTGTAAACTAAAAGCTTCAGGGTTATGTCTATGTTCAATTCTATAGAAGAAA  
 TGCAAATATCACTGTATTTAATATTTGTTATTTCTCTCATGAATAGAAATT  
 TATGTAGAAGCAAACAAAATACTTTTACCCACTTAAAAAGAGAATATAAC  
 ATTTTATGTCACTATAATCTTTTGTTTTAAAGTTAGTGTATATTTTGTGT  
 GATTATCTTTTGTGGTGTGAATAAATCTTTTATCTTGAATGTAATAAGAA  
 TTTGGTGGTGTCAATTGCTTATTTGTTTTCCACGGTTGTCCAGCAATTAAT  
 AAAACATAACCTTTTTTACTGCCTAAAAAATAAAAAAAAAAAAAAAAAAAAA  
 AAAAAAAAAA

SEQ ID NO: 84

**Chondroitin Sulfate Proteoglycan 2**

>gi|21361115|ref|NM\_004385.2| Homo sapiens chondroitin sulfate  
 proteoglycan 2 (versican) (CSPG2), mRNA | qPCR forward\_primer match  
 [10087..10106] | qPCR reverse\_primer match [10185..10163] | qPCR probe match  
 [10139..10161]

GCTGCCCCGAGCCTTTCTGGGGAAGAACTCCAGGCGTGCGGACGCA  
 ACAGCCGAGAACATTAGGTGTTGTGGACAGGAGCTGGGACCAAGATCTTC  
 GGCCAGCCCCGCATCCTCCCGCATCTTCCAGCACCGTCCCGCACCTCCGC  
 ATCCTTCCCCGGGCCACACGCTTCCATATGTGACCCGCCTGGGCAACGCCG  
 AACCCAGTCGCGCAGCGCTGCAGTGAATTTCCCCCAAACCTGCAATAAG  
 CCGCCTTCCAAGGCCAAGATGTTTATAAATATAAAGAGCATCTTATGGAT  
 GTGTTCAACCTTAATAGTAACCCATGCGCTACATAAAGTCAAAGTGGGAA  
 AAAGCCCACCGGTGAGGGGCTCCCTCTCTGGAAAAGTCAGCCTACCTTGT

CATTTTTCAACGATGCCTACTTTGCCACCCAGTTACAACACCAGTGAATTT  
CTCCGCATCAAATGGTCTAAGATTGAAGTGGACAAAAATGGAAAAGATTT  
GAAAGAGACTACTGTCCTTGTGGCCCAAATGGAAATATCAAGATTGGTC  
AGGACTACAAAGGGAGAGTGTCTGTGCCACACATCCCGAGGCTGTGGGC  
GATGCCTCCCTCACTGTGGTCAAGCTGCTGGCAAGTGATGCGGGTCTTTAC  
CGCTGTGACGTCATGTACGGGATTGAAGACACACAAGACACGGTGTCACT  
GACTGTGGATGGGGTTGTGTTTCACTACAGGGCGGCAACCAGCAGGTACA  
CACTGAATTTTGAGGCTGCTCAGAAGGCTTGTTTGACGTTGGGGCAGTC  
ATAGCAACTCCAGAGCAGCTCTTTGCTGCCTATGAAGATGGATTTGAGCA  
GTGTGACGCAGGCTGGCTGGCTGATCAGACTGTCAGATATCCCATCCGGG  
CTCCCAGAGTAGGCTGTTATGGAGATAAGATGGGAAAGGCAGGAGTCAG  
GACTTATGGATTCCGTTCTCCCCAGGAACTTACGATGTGTATTGTTATGT  
GGATCATCTGGATGGTGTGTGTTCCACCTCACTGTCCCCAGTAAATTCAC  
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ACAGTGGGGGAACTCCAGGCGGCATGGAGGAACGGCTTTGACCAGTGCG  
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AGGGCCCAAGTGTGGAGGTGGTCTACTTGGGGTGAGAACCTGTATCGTTT  
TGAGAACCAGACAGGCTTCCCTCCCCCTGATAGCAGATTTGATGCCTACTG  
CTTTAAACCTAAAGAGGCTACAACCATCGATTTGAGTATCCTCGCAGAAA  
CTGCATCACCCAGTTTATCCAAAGAACCACAAATGGTTTCTGATAGAACT  
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TCAGGCTATCACAGATAGTTTAGCCACCAAATTACCCACACCTACTGGCA  
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AGATTTTCAGCCAACTACATCAACTGGTATTGTCAGAAAAGTCAACTTTGAG  
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TGTAGCCAAGGAAACAGAAACCGATATTGATAGAGAGTATTTACGACTT  
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CCTACCAAGTTTGTAAGTGAAACAGACACTTCTGAGTGGATTTCAGTACC



ACTGTTGAGGAAAAGAAAAGGAAGGAGGAGGAGGGAACCTACAGGTACGG  
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CTCTGGAGAAGCTGCTGCCGACCCAGAAACCACCACTGTTTCTTCATTTTC  
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TGTCTCCGCATGTGGAACTACATTCTCCACTGAGCCAACAGGACTGGTTT  
TGAGTACAGTAATGGACAGAGTAGTTGCTGAAAATATAACCCAAACATCC  
AGGGAAATAGTGATTTTCAGAGCGATTAGGAGAACCAAATTATGGGGCAG  
AAATAAGGGGCTTTTCCACAGGTTTTCTTTGGAGGAAGATTTTCAGTGGTG  
ACTTTAGAGAATACTCAACAGTGTCTCATCCCATAGCAAAAGAAGAAACG  
GTAATGATGGAAGGCTCTGGAGATGCAGCATTTAGGGACACCCAGACTTC  
ACCATCTACAGTACCTACTTCAGTTTACATCAGTCACATATCTGACTCAGA  
AGGACCCAGTAGCACCATGGTCAGCACTTCAGCCTTCCCCTGGGAAGAGT  
TTACATCCTCAGCTGAGGGCTCAGGTGAGCAACTGGTCACAGTCAGCAGC  
TCTGTTGTTCCAGTGCTTCCCAGTGCTGTGCAAAAGTTTTCTGGTACAGCT  
TCCTCCATTATCGACGAAGGATTGGGAGAAGTGGGTACTGTCAATGAAAT  
TGATAGAAGATCCACCATTTTACCAACAGCAGAAGTGGAAGGTACGAAAG  
CTCCAGTAGAGAAGGAGGAAGTAAAGGTCAAGTGGCACAGTTTCAACAAA  
CTTTCCCCAAACTATAGAGCCAGCCAAATTATGGTCTAGGCAAGAAGTCA  
ACCCTGTAAGACAAGAAATTGAAAGTGAAACAACATCAGAGGAACAAAT  
TCAAGAAGAAAAGTCATTTGAATCCCCTCAAAACTCTCCTGCAACAGAAC  
AAACAATCTTTGATTCACAGACATTTACTGAAACTGAACTCAAAACCACA  
GATTATTCTGTACTAACAACAAAGAAAACCTTACAGTGATGATAAAGAAAT  
GAAGGAGGAAGACACTTCTTTAGTTAACATGTCTACTCCAGATCCAGATG  
CAAATGGCTTGGAATCTTACACAACCTCTCCCTGAAGCTACTGAAAAGTCA  
CATTTTTTCTTAGCTACTGCATTAGTAACTGAATCTATAACCAGCTGAACAT  
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GAAAGGCATGAGACCAACAATTCAAGAGTCAGATACTGAGCTCTTATTCT  
CTGGACTGGGATCAGGAGAAGAAGTTTTACCTACTCTACCAACAGAGTCA  
GTGAATTTTACTGAAGTGGAACAAATCAATAACACATTATATCCCCACAC  
TTCTCAAGTGGAAGTACCTCAAGTGACAAAATTGAAGACTTTAACAGAA  
TGGAAAATGTGGCAAAAGAAGTTGGACCACTCGTATCTCAAACAGACATC  
TTTGAAGGTAGTGGGTCAGTAACCAGCACAAACATTAATAGAAATTTTAAG  
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ACATCGGACATCCTCAAAATCAGACTGTCAGGTGGGCAGAAGAAATCCAG  
ACTAGTAGACCACAAACCATAACTGAACAAGACTCTAACAAGAATTCTTC  
AACAGCAGAAATTAACGAAACAACAACCTCATCTACTGATTTTTCTGGCTA  
GAGCTTATGGTTTTTGAAATGGCCAAAGAATTTGTTACATCAGCACCAAAA  
CCATCTGACTTGTATTATGAACCTTCTGGAGAAGGATCTGGAGAAGTGGA  
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AAGCAGCACCATTTGTTTTCTGATGGGTCCCTGGAAAAACATCCTGAGG  
TGCCAAGCGCTAAAGCTGTTACTGCTGATGGATTCCCAACAGTTTCAGTGA  
TGCTGCCTCTTCATTCAGAGCAGAACAAAAGCTCCCCTGATCCAACCTAGC  
ACACTGTCAAATACAGTGTCTATATGAGAGGTCCACAGACGGTAGTTTCCA  
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CCACATGACAGTAATGATGAAAGTAATGATGACAGCACTCAAGTTCAAGA  
GATCTATGAGGCAGCTGTCAACCTTTCTTTAACTGAGGAAACATTTGAGG  
GCTCTGCTGATGTTCTGGCTAGCTACACTCAGGCAACACATGATGAATCA  
ATGACTTATGAAGATAGAAGCCAACCTAGATCACATGGGCTTTCACTTCAC  
AACTGGGATCCCTGCTCCTAGCACAGAAACAGAATTAGACGTTTTACTTCC  
CACGGCAACATCCCTGCCAATTCTCGTAAGTCTGCCACAGTTATTCCAGA  
GATTGAAGGAATAAAAGCTGAAGCAAAAGCCCTGGATGACATGTTTGAAT  
CAAGCACTTTGTCTGATGGTCAAGCTATTGCAGACCAAAGTGAAATAATA  
CCAACATTGGGCCAATTTGAAAGGACTCAGGAGGAGTATGAAGACAAAA  
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TATTACACTGATACAACATTAGCAGTTTCAACATTTGCGAAGTTGTCTTCT  
CAGACACCATCATCTCCCTCACTATCTACTCAGGCAGTGAAGCCTCTGGA  
CACACAGAGATCCCCCAGCCCAGTGCTCTGCCAGGAATAGACGTCGGCTC  
ATCTGTAATGTCCCCACAGGATTCTTTTAAGGAAATTCATGTAAATATTGA  
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CCTCTTTATCTCCTGACACAAAATTAGAACCTTCAGAAGATGATGGTAAAC  
CTGAGTTATTAGAAGAAATGGAAGCTTCTCCACAGAACTTATTGCTGTG  
GAAGGAACTGAGATTCTCCAAGATTTCAAAACAAAACCGATGGTCAAGT  
TTCTGGAGAAGCAATCAAGATGTTTCCCAACCATTAACACCTGAGGCTG  
GAACTGTTATTACAACCTGCCGATGAAATTGAATTAGAAGGTGCTACACAG  
TGGCCACACTCTACTTCTGCTTCTGCCACCTATGGGGTCGAGGCAGGTGTG  
GTGCCTTGGCTAAGTCCACAGACTTCTGAGAGGCCACGCTTTCTTCTTCT  
CCAGAAATAAACCTGAACTCAAGCAGCTTTAATCAGAGGGGCAGGATTC  
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CCACCATTTTCCCTTCTGGGAGACTTCTAATGAAACAGATTTCCCTGATTGGC  
ATTAATGAAGAGTCAGTGGAAGGCACGGCAATCTATTTACCAGGACCTGA  
TCGCTGCAAAATGAACCCGTGCCTTAACGGAGGCACCTGTTATCCTACTG  
AACTTCCTACGTATGCACCTGTGTGCCAGGATACAGCGGAGACCAGTGT  
GAACTTGATTTTGATGAATGTCACTCTAATCCCTGTGTAATGGAGCCACT  
TGTGTTGATGGTTTTAACACATTCAGGTGCCTCTGCCTTCCAAGTTATGTT  
GGTGCACCTTTGTGAGCAAGATACCGAGACATGTGACTATGGCTGGCACAA  
ATTCCAAGGGCAGTGCTACAAATACTTTGCCCATCGACGCACATGGGATG  
CAGCTGAACGGGAATGCCGTCTGCAGGGTGCCCATCTCACAAGCATCCTG  
TCTCACGAAGAACAAATGTTTGTTAATCGTGTGGGCCATGATTATCAGTGG  
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GGAATGATGTTCCCTGCAATTACCATCTCACCTATACGTGCAAGAAAGGA  
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AAGATGGTTTCATTCAACGTCACCTTCCAACCTATCCGGTGCTTAGGAAATG  
GAAGATGGGCTATACCTAAAATTACCTGCATGAACCCATCTGCATACCAA  
AGGACTTATTCTATGAAATACTTTAAAAATTCTCATCAGCAAAGGACAA  
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AGTCGAGGCGCTGATCCCTAAAATGGCGAACATGTGTTTTTCATCATTTTCAG  
 CCAAAGTCCTAACTTCCTGTGCCTTTTCTATCACCTCGAGAAGTAATTATC  
 AGTTGGTTTTGGATTTTTGGACCACCGTTCAGTCATTTTGGGTTGCCGTGCT  
 CCCAAAACATTTTAAATGAAAGTATTGGCATTCAAAAAGACAGCAGACAA  
 AATGAAAGAAAATGAGAGCAGAAAGTAAGCATTTCCAGCCTATCTAATTT  
 CTTTAGTTTTCTATTTGCCTCCAGTGCAGTCCATTTCTAATGTATACCAGC  
 CTACTGTACTATTTAAAATGCTCAATTTAGCACCAGATGGCCATGTAAATA  
 AGATGATTTAATGTTGATTTTAATCCTGTATATAAAATAAAAAGTCACAAT  
 GAGTTTGGGCATATTTAATGATGATTATGGAGCCTTAGAGGTCTTTAATCA  
 TTGGTTTCGGCTGCTTTTATGTAGTTTAGGCTGGAAATGGTTTCACTTGCTCT  
 TTGACTGTCAGCAAGACTGAAGATGGCTTTTCTTGACAGCTAGAAAACA  
 CAAAATCTTGATGGTCATTGCACCTATCTCAGCCATAGGTGCAGTTTGCTT  
 CTACATGATGCTAAAGGCTGCGAATGGGATCCTGATGGAACTAAGGACTC  
 CAATGTCGAACTCTTCTTTGCTGCATTCCTTTTCTTCACTTACAAGAAAGG  
 CCTGAATGGAGGACTTTTCTGTAACCAGG

SEQ ID NO: 85

### N-Acylsphingosine Amidohydrosase 1

>gi|30089929|ref|NM\_004315.2| Homo sapiens N-acylsphingosine  
 amidohydrolase (acid ceramidase) 1 (ASAHI), transcript variant 2, mRNA | qPCR  
 forward\_primer match [1212..1228] | qPCR reverse\_primer match [1290..1266] |  
 qPCR probe match [1233..1260]

GGACTTTGAAATCCAACCCGGTCACCTACCCGCGCGACTGTGTCCA  
 CGGATGGCACGAAAGCCAAGCGAGTCCCCCTGCCGAGCTACTCGCGTCCG  
 CCTCCTCCCAAGCTGAGCTCTGCTCCGCCCACCTGAGTCCTTCGCCAGTTA  
 GGAGGAAACACAGCCGCTTAATGAACTGCTGCATCGGGCTGGGAGAGAA  
 AGCTCGCGGGTCCCACCGGGCCTCCTACCCAAGTCTCAGCGCGCTTTTCAC  
 CGAGGCCTCAATTCTGGGATTTGGCAGCTTTGCTGTGAAAGCCCAATGGA  
 CAGAGGACTGCAGAAAATCAACCTATCCTCCTTCAGGACCAACGTACAGA  
 GGTGCAGTTCCATGGTACACCATAAATCTTGACTTACCACCCTACAAAAG  
 ATGGCATGAATTGATGCTTGACAAGGCACCAATGCTAAAGGTTATAGTGA  
 ATTCTCTGAAGAATATGATAAATACATTTCGTGCCAAGTGGAAGGTTATG  
 CAGGTGGTGGATGAAAAATTGCCTGGCCTACTTGGCAACTTTCCTGGCCCT  
 TTTGAAGAGGAAATGAAGGGTATTGCCGCTGTTACTGATATACCTTTAGG  
 AGAGATTATTTCAATTCAATATTTTTTATGAATTATTTACCATTGTACTTCA  
 ATAGTAGCAGAAGACAAAAAAGGTCATCTAATACATGGGAGAAACATGG  
 ATTTTGGAGTATTTCTTGGGTGGAACATAAATAATGATACCTGGGTCATAA  
 CTGAGCAACTAAAACCTTTAACAGTGAATTTGGATTTCCAAAGAAACAAC  
 AAAACTGTCTTCAAGGCTTCAAGCTTTGCTGGCTATGTGGGCATGTTAACA  
 GGATTCAAACCAGGACTGTTCACTTACACTGAATGAACGTTTCAGTATA  
 AATGGTGGTTATCTGGGTATTCTAGAATGGATTCTGGGAAAGAAAGATGC  
 CATGTGGATAGGGTTCCTCACTAGAACAGTTCTGGAAAATAGCACAAGTT  
 ATGAAGAAGCCAAGAATTTATTGACCAAGACCAAGATATTGGCCCCAGCC  
 TACTTTATCCTGGGAGGCAACCAGTCTGGGGAAGGTTGTGTGATTACACG  
 AGACAGAAAGGAATCATTGGATGTATATGAACTCGATGCTAAGCAGGGTA  
 GATGGTATGTGGTACAAACAAATTATGACCGTTGGAAACATCCCTTCTTCC  
 TTGATGATCGCAGAACGCCTGCAAAGATGTGTCTGAACCGCACCCAGCCAA  
 GAGAATATCTCATTTGAAACCATGTATGATGTCCTGTCAACAAAACCTGTC

CTCAACAAGCTGACCGTATACACAACCTTGATAGATGTTACCAAAGGTCA  
 ATTCGAAACTTACCTGCGGGACTGCCCTGACCCTTGTATAGGTTGGTGAGC  
 ACACGTCTGGCCTACAGAATGCGGCCTCTGAGACATGAAGACACCATCTC  
 CATGTGACCGAACACTGCAGCTGTCTGACCTTCCAAAGACTAAGACTCGC  
 GGCAGGTTCTCTTTGAGTCAAAAGCTTGTCTTCGTCCATCTGTTGACAAAT  
 GACAGACCTTTTTTTTTCCCCCATCAGTTGATTTTTCTTATTTACAGATAAC  
 TTCTTTAGGGGAAGTAAACAGTCATCTAGAATTCAGTGGTGGTGAACCTCCACCT  
 CTTTGACATTTGGGGATCTGGTGGGCAGTCGAACCATGGTGAACCTCCACCT  
 CCGTGGAATAAATGGAGATTGAGCGTGGGTGTTGAATCCAGCACGTCTGT  
 GTGAGTAACGGGACAGTAAACACTCCACATTCTTCAGTTTTTCACTTCTAC  
 CTACATATTTGTATGTTTTTCTGTATAACAGCCTTTTCTTCTGGTTCTAAC  
 TGCTGTTAAATAATATATCATTATCTTTGCTGTTATTGACAGCGATATA  
 ATTTTATTACATATGATTAGAGGGATGAGACAGACATTACCTGTATATTT  
 CTTTAAATGGGCACAAAATGGGCCCTTGCCTCTAAATAGCACTTTTTGGGG  
 TTCAAGAAGTAATCAGTATGCAAAGCAATCTTTTATACAATAATTGAAGT  
 GTTCCCTTTTTTCATAATTACTGTACTTCCCAGTAACCCTAAGGAAGTTGCT  
 AACTTAAAAAACTGCATCCCACGTTCTGTTAATTTAGTAAATAAACAAGTC  
 AAAGACTTGTGGAAAATAGGAAGTGAACCCATATTTTAAATTCTCATAAG  
 TAGCATTTCATGTAATAAACAGGTTTTTAGTTTGTCTTCAGATTGATAGGG  
 AGTTTTAAAGAAATTTTAGTAGTTACTAAAATTATGTTACTGTATTTTCA  
 GAAATCAAACCTGCTTATGAAAAGTACTAATAGAACTTGTTAACCTTTCTAA  
 CCTTCACGATTAACCTGTGAAATGTACGTCATTTGTGCAAGACCGTTTGTCC  
 ACTTCATTTTGTATAATCACAGTTGTGTTCTGACACTCAATAAACAGTCA  
 TTGGAAAGAGTGCCAGTCAGCAGTCATGCA

SEQ ID NO: 86

#### N-Acylsphingosine Amidohydrolase 1 Transcript Variant 1

>gi|30089927|ref|NM\_177924.1| Homo sapiens N-acylsphingosine  
 amidohydrolase (acid ceramidase) 1 (ASAH1), transcript variant 1, mRNA | qPCR  
 forward\_primer match [1050..1066] | qPCR reverse\_primer match [1128..1104] |  
 qPCR probe match [1071..1098]

GGCTCTTCTTTGCCTCTGCTGGAGTCCGGGGAGTGGCGTTGGCTGCT  
 AGAGCGATGCCGGGCCGGAGTTGCGTCGCCCTTAGTCCTCCTGGCTGCCGC  
 CGTCAGCTGTGCCGTCGCGCAGCACGCGCCGCGCTGGACAGAGGACTGCA  
 GAAAATCAACCTATCCTCCTTCAGGACCAACGTACAGAGGTGCAGTTCCA  
 TGGTACACCATAAATCTTGACTTACCACCCTACAAAAGATGGCATGAATT  
 GATGCTTGACAAGGCACCAATGCTAAAGGTTATAGTGAATTCTCTGAAGA  
 ATATGATAAATACATTTCGTGCCAAGTGGAAAAGTTATGCAGGTGGTGGAT  
 GAAAAATTGCCTGGCCTACTTGGCAACTTTCCTGGCCCTTTTGAAGAGGAA  
 ATGAAGGGTATTGCCGCTGTTACTGATATACCTTTAGGAGAGATTATTCA  
 TTCAATATTTTTTATGAATTATTTACCATTGTACTTCAATAGTAGCAGAA  
 GACAAAAAAGGTCATCTAATACATGGGAGAAACATGGATTTTGGAGTATT  
 TCTTGGGTGGAACATAAATAATGATACCTGGGTCATAACTGAGCAACTAA  
 AACCTTTAACAGTGAATTTGGATTTCCAAAGAAACAACAAAACCTGTCTTC  
 AAGGCTTCAAGCTTTGCTGGCTATGTGGGCATGTTAACAGGATTCAAACC  
 AGGACTGTTCACTTACACTGAATGAACGTTTCAGTATAAATGGTGGTTA  
 TCTGGGTATTCTAGAATGGATTCTGGGAAAGAAAGATGCCATGTGGATAG  
 GGTTCTCACTAGAACAGTTCTGGAAAATAGCACAAAGTTATGAAGAAGCC

AAGAATTTATTGACCAAGACCAAGATATTGGCCCCAGCCTACTTTATCCTG  
GGAGGCAACCAGTCTGGGGAAGGTTGTGTGATTACACGAGACAGAAAGG  
AATCATTGGATGTATATGAACTCGATGCTAAGCAGGGTAGATGGTATGTG  
GTACAAACAAATTATGACCGTTGGAAACATCCCTTCTTCCTTGATGATCGC  
AGAACGCCTGCAAAGATGTGTCTGAACCGCACCAGCCAAGAGAATATCTC  
ATTTGAAACCATGTATGATGTCCTGTCAACAAAACCTGTCCTCAACAAGCT  
GACCGTATACACAACCTTGATAGATGTTACCAAAGGTCAATTTCGAAACTT  
ACCTGCGGGGACTGCCCTGACCCTTGATAGGTTGGTGAGCACACGTCTGG  
CCTACAGAATGCGGCCTCTGAGACATGAAGACACCATCTCCATGTGACCG  
AACACTGCAGCTGTCTGACCTTCCAAAGACTAAGACTCGCGGCAGGTTCT  
CTTTGAGTCAAAAGCTTGTCTTCGTCCATCTGTTGACAAATGACAGACCTT  
TTTTTTTCCCCCATCAGTTGATTTTCTTATTTACAGATAACTTCTTTAGGG  
GAAGTAAACAGTCATCTAGAATTCAGTGGTGGTTCAGTTTGTTCAGTTGACATT  
TGGGGATCTGGTGGGCAGTCGAACCATGGTGAACCTCCACCTCCGTGGAAT  
AAATGGAGATTGAGCGTGGGTGTTGAATCCAGCACGTCTGTGTGAGTAAC  
GGGACAGTAAACACTCCACATTCTTCAGTTTTTCACTTCTACCTACATATT  
TGTATGTTTTTCTGTATAACAGCCTTTTCTTCTGTTCTAACTGCTGTAA  
AATTAATATATCATTATCTTTGCTGTTATTGACAGCGATATAATTTTATTAC  
ATATGATTAGAGGGATGAGACAGACATTACCTGTATATTTCTTTTAAATGG  
GCACAAAATGGGCCCTTGCCCTCTAAATAGCACTTTTGGGGGTTCAAGAAG  
TAATCAGTATGCAAAGCAATCTTTTATACAATAATTGAAGTGTTCCTTTT  
TCATAATTACTGTACTTCCCAGTAACCCTAAGGAAGTTGCTAACTTAAAAA  
ACTGCATCCCACGTTCTGTAAATTTAGTAAATAAACAAAGTCAAAGACTTGT  
GGAAAATAGGAAGTGAACCCATATTTTAAATTCTCATAAGTAGCATTTCAT  
GTAATAAACAGGTTTTTAGTTTGTCTTCAGATTGATAGGGAGTTTTAAAG  
AAATTTTAGTAGTTACTAAAATTATGTTACTGTATTTTTCAGAAATCAAAC  
TGCTTATGAAAAGTACTAATAGAACTTGTTAACCTTTCTAACCTTCACGAT  
TAACTGTGAAATGTACGTCAATTTGTGCAAGACCGTTTGTCCACTTCATTTT  
GTATAATCACAGTTGTGTTCCCTGACACTCAATAAACAGTCATTGGAAAGA  
GTGCCAGTCAGCAGTCATGCA

SEQ ID NO: 87

**Protease, Serine 11**

>gi|21327712|ref|NM\_002775.2| Homo sapiens protease, serine, 11 (IGF binding) (PRSS11), mRNA | qPCR forward\_primer match [1030..1048] | qPCR reverse\_primer match [1106..1083] | qPCR probe match [1080..1050]

CCGGCCCTCGCCCTGTCCGCCGCCACCGCCGCCGCCGAGAGTCG  
CCATGCAGATCCCGCGCGCCGCTCTTCTCCCGCTGCTGCTGCTGCTGG  
CGGCGCCCGCCTCGGCGCAGCTGTCCCGGGCCGGCCGCTCGGCGCCTTTG  
GCCGCCGGGTGCCAGACCGCTGCGAGCCGGCGCGCTGCCCGCCGACGCC  
GGAGCACTGCGAGGGCGGCCGGGCCGGACGCGTGCGGCTGCTGCGAG  
GTGTGCGGCGCGCCCGAGGGCGCCGCGTGCGGCCTGCAGGAGGGCCCGTG  
CGGCGAGGGGCTGCAGTGCGTGGTGCCCTTCGGGGTGCCAGCCTCGGCCA  
CGGTGCGGCGGCGCGCAGGCCGGCCTCTGTGTGTGCGCCAGCAGCGAG  
CCGGTGTGCGGCAGCGACGCCAACACCTACGCCAACCTGTGCCAGCTGCG  
CGCCGCCAGCCGCCGCTCCGAGAGGCTGCACCGGCCGCCGGTCATCGTCC  
TGCAGCGCGGAGCCTGCGGCCAAGGGCAGGAAGATCCCAACAGTTTGCGC  
CATAAATATAACTTTATCGCGGACGTGGTGGAGAAGATCGCCCTGCCGT  
GGTTCATATCGAATTGTTTCGCAAGCTTCCGTTTTCTAAACGAGAGGTGCC

GGTGGCTAGTGGGTCTGGGTTTATTGTGTCGGAAGATGGACTGATCGTGA  
CAAATGCCACGTGGTGACCAACAAGCACCGGGTCAAAGTTGAGCTGAAG  
AACGGTGCCACTTACGAAGCCAAAATCAAGGATGTGGATGAGAAAGCAG  
ACATCGCACTCATCAAAATTGACCACCAGGGCAAGCTGCCTGTCCTGCTG  
CTTGCCGCTCCTCAGAGCTGCGGCCGGGAGAGTTTCGTGGTTCGCCATCGG  
AAGCCCGTTTTCCCTTCAAAACACAGTCACCACCAGGGATCGTGAGCACC  
CCCAGCGAGGCGGCAAAGAGCTGGGGCTCCGCAACTCAGACATGGACTA  
CATCCAGACCGACGCCATCATCAACTATGGAAACTCGGGAGGCCCGTTAG  
TAAACCTGGACGGTGAAGTGATTGGAATTAACACTTTGAAAGTGACAGCT  
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TCCCATGACCGACAGGCCAAAGGAAAAGCCATCACCAAGAAGAAGTATA  
TTGGTATCCGAATGATGTCACTCACGTCCAGCAAAGCCAAAGAGCTGAAG  
GACCGGCACCGGGACTTCCCAGACGTGATCTCAGGAGCGTATATAATTGA  
AGTAATTCCTGATACCCAGCAGAAGCTGGTGGTCTCAAGGAAAACGACG  
TCATAATCAGCATCAATGGACAGTCCGTGGTCTCCGCCAATGATGTCAGC  
GACGTCAATTAAGGGGAAAGCACCCCTGAACATGGTGGTCCGCAGGGGTA  
ATGAAGATATCATGATCACAGTGATTCCCGAAGAAATTGACCCATAGGCA  
GAGGCATGAGCTGGACTTCATGTTTCCCTCAAAGACTCTCCCGTGGATGAC  
GGATGAGGACTCTGGGCTGCTGGAATAGGACACTCAAGACTTTTGACTGC  
CATTTTGTGTTGTTGAGTGGAGACTCCCTGGCCAACAGAATCCTTCTTGATA  
GTTTGCAGGCAAAACAAATGTAATGTTGCAGATCCGCAGGCAGAAGCTCT  
GCCCTTCTGTATCCTATGTATGCAGTGTGCTTTTTCTTGCCAGCTTGGGCCA  
TTCTTGCTTAGACAGTCAGCATTTGTCTCCTCCTTTAACTGAGTCATCATCT  
TAGTCCAATAATGCAGTCGATACAATGCGTAGATAGAAGAAGCCCCACG  
GGAGCCAGGATGGGACTGGTCGTGTTTGTGCTTTTCTCCAAGTCAGCACCC  
AAAGGTCAATGCACAGAGACCCCGGGTGGGTGAGCGCTGGCTTCTCAAAC  
GGCCGAAGTTGCCTCTTTTAGGAATCTCTTTGGAATTGGGAGCACGATGAC  
TCTGAGTTTGAGCTATTAAAGTACTTCTTACACATTG SEQ ID NO: 88

#### Secreted Frizzled-Related Protein 2

>gi|42656988|ref|XM\_050625.4| Homo sapiens secreted frizzled-related  
protein 2 (SFRP2), mRNA | qPCR forward\_primer match [686..703] | qPCR  
reverse\_primer match [750..728] | qPCR probe match [705..726]

CCGGGTCGGAGCCCCCGGAGCTGCGCGCGGGCTTGCAGCGCCTCG  
CCGCGCTGTCCTCCCGGTGTCCCGCTTCTCCGCGCCCCAGCCGCCGGCTG  
CCAGCTTTTCGGGGCCCCGAGTCGCACCCAGCGAAGAGAGCGGGCCCCGGG  
ACAAGCTCGAACTCCGGCCGCCTCGCCCTTCCCCGGCTCCGCTCCCTCTGC  
CCCCTCGGGGTGCGCGCGCCACGATGCTGCAGGGCCCTGGCTCGCTGCTG  
CTGCTCTTCCTCGCCTCGCACTGCTGCCTGGGCTCGGCGCGCGGGCTCTTC  
CTCTTTGGCCAGCCCGACTTCTCCTACAAGCGCAGCAATTGCAAGCCCATC  
CCTGCCAACCTGCAGCTGTGCCACGGCATCGAATACCAGAACATGCGGCT  
GCCAACCTGCTGGGCCACGAGACCATGAAGGAGGTGCTGGAGCAGGCC  
GGCGCTTGATCCCGCTGGTCATGAAGCAGTGCCACCCGGACACCAAGAA  
GTTCTGTGCTCGCTCTTCGCCCCCGTCTGCCTCGATGACCTAGACGAGAC  
CATCCAGCCATGCCACTCGCTCTGCGTGCAGGTGAAGGACCGCTGCGCCC  
CGGTCATGTCCGCCTTCGGCTTCCCCTGGCCCGACATGCTTGAGTGCGACC  
GTTTCCCCCAGGACAACGACCTTTGCATCCCCCTCGCTAGCAGCGACCACC  
TCCTGCCAGCCACCGAGGAAGCTCCAAAGGTATGTGAAGCCTGCAAAAAT

AAAAATGATGATGACAACGACATAATGGAAACGCTTTGTAAAAATGATTT  
 TGCACTGAAAATAAAAAGTGAAGGAGATAACCTACATCAACCGAGATACC  
 AAAATCATCCTGGAGACCAAGAGCAAGACCATTACAAAGCTGAACGGTGT  
 GTCCGAAAGGGACCTGAAGAAATCGGTGCTGTGGCTCAAAGACAGCTTGC  
 AGTGCACCTGTGAGGAGATGAACGACATCAACGCGCCCTATCTGGTCATG  
 GGACAGAAACAGGGTGGGGAGCTGGTGATCACCTCGGTGAAGCGGTGGC  
 AGAAGGGGCGAGAGAGAGTTCAAGCGCATCTCCCGCAGCATCCGCAAGCT  
 GCAGTGCTAGTCCCGGCATCCTGATGGCTCCGACAGGCCTGCTCCAGAGC  
 ACGGCTGACCATTTCTGCTCCGGGATCTCAGCTCCCGTTCCCCAAGCACAC  
 TCCTAGCTGCTCCAGTCTCAGCCTGGGCAGCTTCCCCCTGCCTTTTGCACG  
 TTTGCATCCCCAGCATTTCTGAGTTATAAGGCCAAGGAGTGGATAGCTG  
 TTTTCACCTAAAGGAAAAGCCACCCGAATCTTGTAGAAATATTCAAACCT  
 AATAAAATCATGAATATTTTATGAAGTTTAAAAA SEQ ID NO: 89

### Phospholipase A2, Group XIIB

>gi|45505134|ref|NM\_032562.2| Homo sapiens phospholipase A2, group  
 XIIB (PLA2G12B), mRNA

TGTCCCTGGAATTCTGGGACACTGGCTGGGGTTTGAGGAGAGAAGC  
 CAGTACCTACCTGGCTGCAGGATGAAGCTGGCCAGTGGCTTCTTGGTTTTG  
 TGGCTCAGCCTTGGGGGTGGCCTGGCTCAGAGCGACACGAGCCCTGACAC  
 GGAGGAGTCTTATTCAGACTGGGGCCTTCGGCACCTCCGGGGAAGCTTTG  
 AATCCGTCAATAGCTACTTCGATTCTTTCTGGAGCTGCTGGGAGGGAAGA  
 ATGGAGTCTGTGAGTACAGGTGCCGATATGGAAAGGCACCAATGCCCCAGA  
 CCTGGCTACAAGCCCCAAGAGCCCCAATGGCTGCGGCTCCTATTTCTGGGT  
 CTCAAGGTACCAGAAAGTATGGACTTGGGCATTCCAGCAATGACAAAGTG  
 CTGCAACCAGCTGGATGTCTGTTATGACACTTGCAGGTGCCAACAAATATC  
 GCTGTGATGCAAAATTCGATGGTGTCTCCACTCGATCTGCTCTGACCTTA  
 AGCGGAGTCTGGGCTTTGTCTCCAAAGTGGAAGCAGCCTGTGATTCCCTG  
 GTTGACACTGTGTTCAACACCGTGTGGACCTTGGGCTGCCGCCCTTTATG  
 AATAGTCAGCGGGCAGCTTGCATCTGTGCAGAGGAGGAGAAGGAAGAGT  
 TATGAGGAAGAAGTGATTCCTTCCTGGTTTTTGAGTGACACCACAGCTGTCA  
 GCCTTCAAGATGTCAAGTCTTCGAGTCAGCGTGAATTCATTCTTCCAA  
 CAGTTTGGACACCACAAAGCAGGAGAAAGGGAACATTTTTCTACAGCTGG  
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 TGAAAGCTACTCTTCATTTAAGACTGCTCTCCCCAACCAAGACACATTTGC  
 CTGGAATTCAGTTCTTAGCTTAAAGACTAAAATGCAAGCAAACCTGCA  
 ATTCCTGGACCTGATAGTTATATTCATGAGTGAAATTGTGGGGAGTCCAGC  
 CATTTGGGAGGCAATGACTTTCTGCTGGCCCATGTTTCAGTTGCCAGTAAG  
 CTTCTCACATTTAATAAAGTGTAATTTTATAGAACATT SEQ ID NO: 90

### Spondin 2, Extracellular Matrix Protein

>gi|6912681|ref|NM\_012445.1| Homo sapiens spondin 2, extracellular matrix  
 protein (SPON2), mRNA

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SEQ ID NO: 91

### Olfactomedin 1, Transcript Variant 3

>gi|34335282|ref|NM\_058199.2| Homo sapiens olfactomedin 1 (OLFM1),  
 transcript variant 3, mRNA

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### Thrombospondin Repeat Containing 1

>gi|38016903|ref|NM\_019032.2| Homo sapiens thrombospondin repeat  
 containing 1 (TSRC1), mRNA

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SEQ ID NO: 93

### Thrombospondin 2

>gi|40317627|ref|NM\_003247.2| Homo sapiens thrombospondin 2 (THBS2),  
mRNA | qPCR forward\_primer match [3558..3580] | qPCR reverse\_primer match  
[3682..3655] | qPCR probe match [3597..3623]

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SEQ ID NO: 94

**Adlican**

>gi|18390318|ref|NM\_015419.1| Homo sapiens adlican (DKFZp564I1922),  
 mRNA | qPCR assay\_on\_demand\_context match [694..718]

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CACCACCTTTTCCTGCTGTTTCTCCCCCTCAGCATCTCCTGTGCAGACAGT  
AACCAGTGCTGAAGAATCCTCAGCAGATGTACCTCTACTTGGTGAAGAAG  
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CACAATGGAGTTATTCTTGTGTAACCTGAAGTAACAAGCACACCTCTGGA  
GGAAGTTGTTGATGACCTTTCTGAGAAGACTGAGGAGATAACTTCCACTG  
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GAACCATCTCCTACTCTGCACACATTAGACACAGTCTATGAAAAGCCCAC  
CCATGAAGAGACGGCAACAGAGGGTTGGTCTGCAGCAGATGTTGGATCGT  
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TCACAACCAGATGAGGATAAGATGAAAGAAGACACCTTTGCACACCTTAC  
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GGGAGAAGGAGATTACGCCCCAACAAATTCCGCCACCGGCACAAGCAAA  
CCCCACCCACAACCTTTTGGCCCCATCAGAGACTTTTTCTACTCAACCAACTC  
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CATAAAAGTGACATTTTAGTCACTGGTGAATCAATTACTAATGCCATACCA  
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TCCTCTCCCATTTGCACATGTCCAAACCCAGCATTCTAGTAAGTTTACTGA  
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TGCTTTCGGTCACCGTGCAGCAACCTCAAATCCTAGCCTCCCACTACCAGG



ACGTCAC TGTCTACCTGGGAGACACCAT TGCAATGGAGTGTCTGGCCAAA  
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GCAAAC TGTGTCCCCCGTGGAGAGCCGCATCACCTGCACGAAAACCGGA  
CCCTTTT CCATCAAGGAGGCGTCTTCTCAGACAGAGGCGTCTATAAGTGC  
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CAGCCA AC GCGCGCATCACGGGCACCTCCCCGCGGAGGACGGACGTGAG  
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GCCAAG ATTGAACACAAGGAGGAGAACGACCACAAAGTCTTCTACGGGG  
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TGACAG CGGTGGACGCACCAAGCGCTATGTCGTCTTCAACAATGGGACAC  
TCTACTT TAACGAAGTGGGGATGAGGGAGGAAGGAGACTACACCTGCTTT  
GCTGAAA ATCAGGTCGGGAAGGACGAGATGAGAGTCAGAGTCAAGGTGG  
TGACAG CGCCCCGCCACCATCCGGAACAAGACTTACTTGGCGGTTTCAGGTG  
CCCTAT GGAGACGTGGTCACTGTAGCCTGTGAGGCCAAAGGAGAACCCAT  
GCCCAAG GTGACTTGGTTGTCCCCAACCAACAAGGTGATCCCCACCTCCTC  
TGAGAAG TATCAGATATACCAAGATGGCACTCTCCTTATTTCAGAAAGCCC  
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GAGGAT AGGAAGACGGTGTGGATTACGTCAACGTCCAGCCACCCAAGAT  
CAACGGT AACCCCAACCCCATCACACCGTGCGGGAGATAGCAGCCGGGG  
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GCACCC CTCCCGGGGCTGGGCAGGGACGTTTCTCCTGGACGCTCCCCAAT  
GGCATGC ATCTGGAGGGCCCCCAAACCCTGGGACGCGTTTCTCTTCTGGA  
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 GACAAGTCATCTTTCAGTTATTTCTCTGTCACTTCAAACTCCAGCTTGC  
 CCAATAAGGATTTAGAACCAGAGTGACTGATATATATATATATATTTTAAAT  
 TCAGAGTTACATACATACAGCTACCATTTTATATGAAAAAAGAAAAACAT  
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 TCAAATCAGACGATGAGACTAGAAGGAGAAATACTTTCTGTCTTATTAAA  
 ATTAATAAATTATTGGTCTTTACAAGACTTGGATACATTACAGCAGACATG  
 GAAATATAAATTTTAAAAAATTTCTCTCCAACCTCCTTCAAATTCAGTCACC  
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 GAGAGGAGAGGAAGGAGAAAACCTGCATCATAACTTTACAGAATTGAATC  
 TAGAGTCTTCCCCGAAAAGCCAGAACTTCTCTGCAGTATCTGGCTTGTC  
 CATCTGGTCTAAGGTGGCTGCTTCTTCCCCAGCCATGAGTCAGTTTGTGCC  
 CATGAATAATACACGACCTGTTATTTCCATGACTGCTTTACTGTATTTTA  
 AGGTCAATATACTGTACATTTGATAATAAAATAATATTCTCCCAAAAAAA  
 AAA

SEQ ID NO: 95

### Cystatin SA

>gi|19882252|ref|NM\_001322.2| Homo sapiens cystatin SA (CST2), mRNA |  
 qPCR forward\_primer match [302..320] | qPCR reverse\_primer match [393..370] |  
 qPCR probe match [341..369]

GCCTCCGAGGAGACCATGGCCTGGCCCCTGTGCACCCTGCTGCTCC  
 TGCTGGCCACCCAGGCTGTGGCCCTGGCCTGGAGCCCCAGGAGGAGGAC  
 AGGATAATCGAGGGTGGCATCTATGATGCAGACCTCAATGATGAGCGGGT  
 ACAGCGTGCCCTTCACTTTGTCATCAGCGAGTATAACAAGGCCACTGAAG  
 ATGAGTACTACAGACGCCTGCTGCGGGTGCTACGAGCCAGGGAGCAGATC  
 GTGGGCGGGGTGAATTACTTCTTCGACATAGAGGTGGGCCGAACCATATG  
 TACCAAGTCCCAGCCCAACTTGGACACCTGTGCCTTCCATGAACAGCCAG  
 AACTGCAGAAGAAACAGTTGTGCTCTTTCCAGATCTACGAAGTTCCCTGG  
 GAGGACAGAATGTCCCTGGTGAATTCCAGGTGTCAAGAAGCCTAGGGATC  
 TGTGCCAGGGAGTCACACTGACCACCTCCTACTCCCACCCCTTGTAGTGCT  
 CCCACCCCTGGACTGGTGGCCCCCACCCTGTGGGAGGTCTCCCATGCACC  
 TGCAGCAGGAGAAGACAGAGAAGGCTGCAGGAGGCCTTTGTTGCTCAGC  
 AGGGGACTCTGCCCTCCCTCCTTCTTTTGTCTTCATAGCCCTGGTACATG  
 GTACACACACCCCCACCTCCTGCAATTAAACAGTAGCATCACCTC

SEQ ID NO: 96

**Cystatin SN**

>gi|19882250|ref|NM\_001898.2| Homo sapiens cystatin SN (CST1), mRNA |  
 qPCR forward\_primer match [358..376] | qPCR reverse\_primer match [449..426] |  
 qPCR probe match [397..425]

GGGCTCCCTGCCTCGGGCTCTCACCTCCTCTCCTGCAGCTCCAGCT  
 TTGTGCTCTGCCTCTGAGGAGACCATGGCCCAGTATCTGAGTACCCTGCTG  
 CTCCTGCTGGCCACCCTAGCTGTGGCCCTGGCCTGGAGCCCCAAGGAGGA  
 GGATAGGATAATCCCGGGTGGCATCTATAACGCAGACCTCAATGATGAGT  
 GGGTACAGCGTGCCCTTCACTTCGCCATCAGCGAGTATAACAAGGCCACC  
 AAAGATGACTACTACAGACGTCCGCTGCGGGTACTAAGAGCCAGGCAACA  
 GACCGTTGGGGGGGTGAATTACTTCTTCGACGTAGAGGTGGGCCGCACCA  
 TATGTACCAAGTCCCAGCCCCAAGTTGGACACCTGTGCCTTCCATGAACAGC  
 CAGAACTGCAGAAGAAACAGTTGTGCTCTTTTCGAGATCTACGAAGTTCCC  
 TGGGAGAACAGAAGGTCCCTGGTGAAATCCAGGTGTCAAGAATCCTAGGG  
 ATCTGTGCCAGGCCATTCGCACCAGCCACCACCCACTCCCACCCCTGTAG  
 TGCTCCACCCCTGGACTGGTGGCCCCCACCCTGCGGGAGGCCTCCCCATG  
 TGCTTGCGCCAAGAGACAGACAGAGAAGGCTGCAGGAGTCCTTTGTGCT  
 CAGCAGGGCGCTCTGCCCTCCCTCCTTCTTCTTCTTAATAGCCCTGGT  
 ACATGGTACACACCCCCCACCTCCTGCAATTAACAGTAGCATCGCCTCC  
 CTCTGAAAAAAAAAAAAAAAAAAAAAAAAA  
 SEQ ID NO: 97

**Lysyl Oxidase-Like Enzyme 2**

>gi|4505010|ref|NM\_002318.1| Homo sapiens lysyl oxidase-like 2 (LOXL2),  
 mRNA | qPCR forward\_primer match [2205..2223] | qPCR reverse\_primer match  
 [2286..2269] | qPCR probe match [2261..2229]

ACTCCAGCGCGCGGCTACCTACGCTTGGTGCTTGCTTTCTCCAGCCA  
 TCGGAGACCAGAGCCGCCCTCTGCTCGAGAAAGGGGCTCAGCGGCGGC  
 GGAAGCGGAGGGGGACCACCGTGGAGAGCGCGGTCCCAGCCCGGCCACT  
 GCGGATCCCTGAAACCAAAAAGCTCCTGCTGCTTCTGTACCCCGCCTGTCC  
 CTCCAGCTGCGCAGGGCCCTTCGTGGGATCATCAGCCCGAAGACAGGG  
 ATGGAGAGGCCTCTGTGCTCCACCTCTGCAGCTGCCTGGCTATGCTGGCC  
 CTCCTGTCCCCCTGAGCCTGGCACAGTATGACAGCTGGCCCCATTACCCC  
 GAGTACTTCCAGCAACCGGCTCCTGAGTATCACCAGCCCCAGGCCCCCGC  
 CAACGTGGCCAAGATTTCAGCTGCGCCTGGCTGGGCAGAAGAGGAAGCAC  
 AGCGAGGGCCGGGTGGAGGTGTACTATGATGGCCAGTGGGGCACCGTGTG  
 CGATGACGACTTCTCCATCCACGCTGCCACGTCGTCTGCCGGGAGCTGG  
 GCTATGTGGAGGCCAAGTCCTGGACTGCCAGCTCCTCCTACGGCAAGGGA  
 GAAGGGCCCATCTGGTTAGACAATCTCCACTGTACTGGCAACGAGGCGAC  
 CCTTGCAGCATGCACCTCCAATGGCTGGGGCGTCACTGACTGCAAGCACA  
 CGGAGGATGTGGTGTGGTGTGCAGCGACAAAAGGATTCTGGGTTCAAA  
 TTTGACAATTCGTTGATCAACCAGATAGAGAACCTGAATATCCAGGTGGA  
 GGACATTCGGATTTCGAGCCATCCTCTCAACCTACCGCAAGCGCACCCAG  
 TGATGGAGGGCTACGTGGAGGTGAAGGAGGGCAAGACCTGGAAGCAGAT  
 CTGTGACAAGCACTGGACGGCCAAGAATTCCCGCGTGGTCTGCGGCATGT  
 TTGGCTTCCCTGGGGAGAGGACATAACAATACCAAAGTGTACAAAATGTTT  
 GCCTCACGGAGGAAGCAGCGCTACTGGCCATTCTCCATGGACTGCACCGG

CACAGAGGCCACATCTCCAGCTGCAAGCTGGGCCCCCAGGTGTCACTGG  
ACCCCATGAAGAATGTACCTGCGAGAATGGGCTGCCGGCCGTGGTGAGT  
TGTGTGCCTGGGCAGGTCTTCAGCCCTGACGGACCCTCGAGATTCCGGAA  
AGCATACAAGCCAGAGCAACCCCTGGTGCGACTGAGAGGCGGTGCCTACA  
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GGGATCGGACCCATCCACCTCAACGAGATCCAGTGCACAGGCAATGAGAA  
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AGGAGGATGCTGGTGTGAGATGCAACACCCCTGCCATGGGCTTGCAAGAAG  
AAGCTGCGCCTGAACGGCGGCCGCAATCCCTACGAGGGCCGAGTGGAGGT  
GCTGGTGGAGAGAAACGGGTCCCTTGTGTGGGGGATGGTGTGTGGCCAAA  
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TTCGCCAGCAACGCCTTCCAGGAGACCTGGTATTGGCACGGAGATGTCAA  
CAGCAACAAAGTGGTCATGAGTGGAGTGAAGTGCTCGGGAACGGAGCTG  
TCCCTGGCGCACTGCCGCCACGACGGGGAGGACGTGGCCTGCCCCCAGGG  
CGGAGTGCAGTACGGGGCCGAGTTGCCTGCTCAGAAACCGCCCCTGACC  
TGGTCTCAATGCGGAGATGGTGCAGCAGACCACCTACCTGGAGGACCGG  
CCCATGTTTCATGCTGCAGTGTGCCATGGAGGAGAACTGCCTCTCGGCCTCA  
GCCGCGCAGACCGACCCACACGGGCTACCGCCGGCTCCTGCGCTTCTC  
CTCCAGATCCACAACAATGGCCAGTCCGACTTCCGGCCCAAGAACGGCC  
GCCACGCGTGGATCTGGCACGACTGTCACAGGCACTACCACAGCATGGAG  
GTGTTCACCCACTATGACCTGCTGAACCTCAATGGCACCAAGGTGGCAGA  
GGGCCACAAGGCCAGCTTCTGCTTGGAGGACACAGAATGTGAAGGAGAC  
ATCCAGAAGAATTACGAGTGTGCCAACTTCGGCGATCAGGGCATCACCAT  
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AGCCGCTATGACGGCCACCGCATCTGGATGTACAACTGCCACATAGGTGG  
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GGGATGAGGCCCACAGACAGGTTGTTCATCAGCTTGTCCCATTCAAGCCAC  
CGAGCTCACCACAGACACAGTGGAGCCGCGCTCTTCTCCAGTGACACGTG  
GACAAATGCGGGCTCATCAGCCCCCCCAGAGAGGGTCAGGCCGAACCCCA  
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TCCAATGAAACCCTCCAGTCTATTATAGTCACATAGATAATGGTGCCACGT  
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ACCCCTTGTTTTTCAAGATACTATTATTATTTTCACAGACTTTTGAAGCA  
CAAATTTATTGGCATTTAATATTGGACATCTGGGCCCTTGGAAGTACAAAT  
CTAAGGAAAAACCAACCCACTGTGTAAGTGAATCATCTTCTGTTGTTCCA  
ATTCTGTGGGTTTTTGATTCAACGGTGCTATAACCAGGGTCCTGGGTGACA  
GGGCGCTCACTGAGCACCATGTGTTCATCACAGACACTTACACATACTTGA  
AACTTGGAATAAAAGAAAGATTTATG

SEQ ID NO: 98

**Thyroglobulin**

&gt;gi|33589851|ref|NM\_003235.3| Homo sapiens thyroglobulin (TG), mRNA |

qPCR forward\_primer match [886..905] | qPCR reverse\_primer match [962..941] |

qPCR probe match [915..939]

GCAGTGGTTTCTCCTCCTTCCTCCCAGGAAGGGCCAGGAAAATGGC  
CCTGGTCCTGGAGATCTTCACCCTGCTGGCCTCCATCTGCTGGGTGTCGGC  
CAATATCTTCGAGTACCAGGTTGATGCCAGCCCCCTTCGTCCCTGTGAGCT  
GCAGAGGGAAACGGCCTTTCTGAAGCAAGCAGACTACGTGCCCCAGTGTG  
CAGAGGATGGCAGCTTCCAGACTGTCCAGTGCCAGAACGACGGCCGCTCC  
TGCTGGTGTGTGGGTGCCAACGGCAGTGAAGTGCTGGGCAGCAGGCAGCC  
AGGACGGCCTGTGGCTTGTCTGTCAATTTTGTCTAGCTACAGAAACAGCAGA  
TCTTACTGAGTGGCTACATTAACAGCACAGACACCTCCTACCTCCCTCAGT  
GTCAGGATTCAGGGGACTACGCGCCTGTTCAAGTGTGATGTGCAGCATGTC  
CAGTGCTGGTGTGTGGACGCAGAGGGGATGGAGGTGTATGGGACCCGCCA  
GCTGGGGAGGCCAAAGCGATGTCCAAGGAGCTGTGAAATAAGAAATCGT  
CGTCTTCTCCACGGGGTGGGAGATAAGTCAACACCCAGTGTTCTGCGGA  
GGGAGAGTTTATGCCTGTCCAGTGCAAATTTGTCAACACCACAGACATGA  
TGATTTTTGATCTGGTCCACAGCTACAACAGGTTTCCAGATGCATTTGTGA  
CCTTCAGTTCCCTCCAGAGGAGGTTCCCTGAGGTATCTGGGTATTGCCACT  
GTGCTGACAGCCAAGGGCGGGAAGTGGCTGAGACAGGTTTGGAGTTGTGA  
CTGGATGAAATTTATGACACCATTTTGTCTGGCCTGGACCTTCCTTCCACC  
TTCAGTCAAACACCCCTGTACCGGATACTGCAGAGACGGTTCCTCGCAGTT  
CAATCAGTCATCTCTGGCAGATTCCGATGCCCCACAAAATGTGAAGTGGA  
GCGGTTTACAGCAACCAGCTTTGGTCAACCCCTATGTTCCAAGCTGCCGCCG  
AAATGGCGACTATCAGGCGGTGCAGTGCCAGACGGAAGGGCCCTGCTGGT  
GTGTGGACGCCCAGGGGAAGGAAATGCATGGAACCCGGCAGCAAGGGGA  
GCCGCCATCTTGTGCTGAAGGCCAATCTTGTGCCTCCGAAAGGCAGCAGG  
CCTTGTCCAGACTCTACTTTGGGACCTCAGGCTACTTCAGCCAGCACGACC  
TGTTCTCTTCCCCAGAGAAAAGATGGGCCTCTCCAAGAGTAGCCAGATTT  
GCCACATCCTGCCCACCCACGATCAAGGAGCTCTTTGTGGACTCTGGGCTT  
CTCCGCCCAATGGTGGAGGGACAGAGCCAACAGTTTTCTGTCTCAGAAAA  
TCTTCTCAAAGAAGCCATCCGAGCAATTTTCCCTCCCGAGGGCTGGCTCG  
TCTTGCCCTTCAGTTTACCACCAACCCAAAGAGACTCCAGCAAAACCTTTT  
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TGGCACAAGAGGCACATTTAACTTCAGTCAATTTTCCAGCAACTTGGTCT  
TGCAAGCTTCTTGAATGGAGGGAGACAAGAAGATTTGGCCAAGCCACTCT  
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TGCAAGATGTCCCCTAGCAGCACTGGAAGGGAAACGGCCCCAGCCCAG  
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AGAAATCTCGAACCAGTGGGCTGCTTTCAGTTGGAAACAGGCTAGATCC  
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CAGCCCAGGCTATGTCCCAGCCTGCAGGGCAGAGGATGGGGGCTTTTCCC  
CAGTGCAATGTGACCAGGCCCAGGGCAGCTGCTGGTGTGTGCATGGACAGC  
GGAGAAGAGGTGCCTGGGACGCGCGTGACCGGGGGCCAGCCCGCCTGTG  
AGAGCCCGCGGTGTCCGCTGCCATTCAACGCGTCGGAGGTGGTTGGTGG  
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GCACTTTCAGCTCCAGCTCCCGCCGGGCAAGATGTGCAGTGCTGACTACG  
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ATCTGTGGGTTGCTGAGCTCACCCAGTGCTCCTGCTTTGTAATGTCAAAGAC  
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CATCAAGAGTCTGACACCCTTAGAAGGAACTCAAGACACCTTTACCAATT  
TTCAGCAGGTTTATCTCTGGAAAGATTCTGACATGGGGTCTCGGCCTGAGT  
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TACTTCACCTGCACCCTCTACCCAGAGGCACAGGTGTGTGATGACATCATG  
GAGTCCAATACCCAGGGCTGCAGACTGATCCTGCCTCAGATGCCAAAGGC  
CCTGTTCCGGAAGAAAGTTATACTGGAAGATAAAGTGAAGAACTTTTACA  
CTCGCCTGCCGTTCCAAAAACTGATGGGGATATCCATTAGAAATAAAGTG  
CCCATGTCTGAAAAATCTATTTCTAATGGGTTCTTTGAATGTGAACGACGG  
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TGCCACCAGCAATTTCTCTGCTGTCCGAGACCTCTGTTTGTGCGAATGTTT  
CCAACATGAGGCCTGTCTCATCACCCTCTGCAAACCCAACTCGGGGCTG  
TGAGATGTATGTTCTATGCTGATACTCAAAGCTGCACACATAGTCTGCAGG  
GTCCGAACTGCCGACTTCTGCTTCGTGAAGAGGCCACCCACATCTACCGG  
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TACCTCATGGAAGCAAGTGGACCAGTTCCTTGGAGTTCCATATGCTGCCCC  
GCCCCTGGCAGAGAGGCACTTCCAGGCACCAGAGCCCTTGAACCTGGACAG  
GCTCCTGGGATGCCAGCAAGCCAAGGGCCAGCTGCTGGCAGCCAGGCACC  
AGAACATCCACGTCTCCTGGAGTCAGTGAAGATTGTTTGTATCTCAATGTG  
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CCCAACTTTTCCGGAGAGCTGTGCTGATGGGAGGCTCCGCACTCTCCCCGG  
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 CCGCCAGAAGCCTGCCAATGTCCTCAATGATGCCAGACCAAGCTCCTGG  
 CCGTGAGTGGCCCTTTCCACTACTGGGGTCTGTGATCGATGGCCACTTCC  
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 GGCTGTGAAGCAATTTGAGGAAAGTCGAGGCCGACCAGTAGCAAAACA  
 GCCTTTTACCAGGCACTGCAGAATTCTCTGGGTGGCGAGGACTCAGATGC  
 CCGCGTCGAGGCTGCTGCTACATGGTATTACTCTCTGGAGCACTCCACGGA  
 TGACTATGCCTCCTTCTCCCGGGCTCTGGAGAATGCCACCCGGGACTACTT  
 TATCATCTGCCCTATAATCGACATGGCCAGTGCCTGGGCAAAGAGGGCCC  
 GAGGAAACGTCCTTCATGTACCATGCTCCTGAAAACCTACGGCCATGGCAGC  
 CTGGAGCTGCTGGCGGATGTTCACTTTGCCTTGGGGCTTCCCTTCTACCCA  
 GCCTACGAGGGGCGAGTTTCTCTGGAGGAGAAGAGCCTGTGCTGAAAAT  
 CATGCAGTACTTTTCCCACTTCATCAGATCAGGAAATCCCAACTACCCTTA  
 TGAGTTCTCACGGAAAGTACCCACATTTGCAACCCCTGGCCTGACTTTGT  
 ACCCGTGCTGGTGGAGAGAACTACAAGGAGTTCAGTGAGCTGCTCCCA  
 ATCGACAGGGCCTGAAGAAAGCCGACTGCTCCTTCTGGTCCAAGTACATC  
 TCGTCTCTGAAGACATCTGCAGATGGAGCCAAGGGCGGGCAGTCAGCAGA  
 GAGTGAAGAGGAGGAGTTGACGGCTGGATCTGGGCTAAGAGAAGATCTC  
 CTAAGCCTCCAGGAACCAGGCTCTAAGACCTACAGCAAGTGACCAGCCCT  
 TGAGCTCCCCAAAAACCTCACCCGAGGCTGCCCACTATGGTCATCTTTTTC  
 TCTAAATAGTTACTTACCTTCAATAAAGTATCTACATGCGGTG

SEQ ID NO: 99

**Transforming Growth Factor, Beta 1**

>gi|10863872|ref|NM\_000660.1| Homo sapiens transforming growth factor,  
 beta 1 (Camurati-Engelmann disease) (TGFB1), mRNA | qPCR forward\_primer  
 match [1651..1668] | qPCR reverse\_primer match [1539..1557] | qPCR probe match  
 [1687..1713]

ACCTCCCTCCGCGGAGCAGCCAGACAGCGAGGGCCCCGGCCGGGG  
 GCAGGGGGGACGCCCCGTCCGGGGACCCCCCGGCTCTGAGCCGCCCG  
 CGGGGCCGGCCTCGGCCCGGAGCGGAGGAAGGAGTCGCCGAGGAGCAGC  
 CTGAGGCCCCAGAGTCTGAGACGAGCCGCCGCCGCCCGCCACTGCGGG  
 GAGGAGGGGGAGGAGGAGCGGGAGGAGGGACGAGCTGGTCGGGAGAAG  
 AGGAAAAAAACTTTTGAGACTTTTCCGTTGCCGCTGGGAGCCGGAGGCGC  
 GGGGACCTCTTGGCGCGACGCTGCCCGCGAGGAGGCAGGACTTGGGGAC  
 CCCAGACCGCCTCCCTTTGCCGCCGGGGACGCTTGCTCCCTCCCTGCCCCC  
 TACACGGCGTCCCTCAGGCGCCCCCATTCGGGACCAGCCCTCGGGAGTCG  
 CCGACCCGGCCTCCCGCAAAGACTTTTCCCCAGACCTCGGGCGCACCCCCCT  
 GCACGCCGCCTTCATCCCCGGCCTGTCTCCTGAGCCCCCGCGCATCCTAGA  
 CCCTTTCTCCTCCAGGAGACGGATCTCTCTCCGACCTGCCACAGATCCCT  
 ATTCAAGACCACCCACCTTCTGGTACCAGATCGCGCCCATCTAGGTTATTT  
 CCGTGGGATACTGAGACACCCCCGGTCCAAGCCTCCCTCCACCACTGCG  
 CCCTTCTCCCTGAGGAGCCTCAGCTTTCCCTCGAGGCCCTCCTACCTTTTGC  
 CGGGAGACCCCCAGCCCCTGCAGGGGGCGGGGCTCCCCACCACACAGCC



CTGTTTCGCGCTCTCGGCAGTGCCGGGGGGCGCCGCCTCCCCCATGCCGCCC  
TCCGGGCTGCGGCTGCTGCCGCTGCTGCTACCGCTGCTGTGGCTACTGGTG  
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CATGGAGCTGGTGAAGCGGAAGCGCATCGAGGCCATCCGCGGCCAGATCC  
TGTCCAAGCTGCGGCTCGCCAGCCCCCGAGCCAGGGGGAGGTGCCGCCC  
GGCCCCGCTGCCCGAGGCCGTGCTCGCCCTGTACAACAGCACCCGCGACCG  
GGTGGCCGGGGAGAGTGCAGAACCAGGAGCCCGAGCCTGAGGCCGACTAC  
TACGCCAAGGAGGTACCCGCGTGCTAATGGTGGAAACCCACAACGAAAT  
CTATGACAAGTTCAAGCAGAGTACACACAGCATATATATGTTCTTCAACA  
CATCAGAGCTCCGAGAAGCGGTACCTGAACCCGTGTTGCTCTCCCGGGCA  
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GCTGTACCAGAAATACAGCAACAATTCCTGGCGATACCTCAGCAACCGGC  
TGCTGGCACCCAGCGACTCGCCAGAGTGGTTATCTTTTGATGTCACCGGAG  
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CAACGGGTTCACTACCGGCCGCCGAGGTGACCTGGCCACCATTCATGGCA  
TGAACCGGCCTTTCCTGCTTCTCATGGCCACCCCGCTGGAGAGGGCCCAGC  
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AGCTCCACGAGAGAAGAACTGCTGCGTGCGGCAGCTGTACATTGACTTCCG  
CAAGGACCTCGGCTGGAAGTGGATCCACGAGCCCAAGGGCTACCATGCCA  
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GCAAGGTCCTGGCCCTGTACAACCAGCATAACCCGGGGCGCCTCGGCGGCG  
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GCAAGTGCAGCTGAGGTCCCGCCCCGCCCCGCCCCGCCCCGGCAGGCCCG  
GCCCCACCCCGCCCCGCCCCCGCTGCCTTGCCCATGGGGGCTGTATTTAAG  
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GTTCCCCCACTCCCACTCCCTCTCTCTCCCTCTCTGCCTCCTCCTGCCTGTC  
TGCACTATTCTTTGCCCCGGCATCAAGGCACAGGGGACCAGTGGGGAAACA  
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TTACATTAATGAACTCATTCAGTCACCATAGCAAACTCTGAGATGGCAG  
GGACTCTGATAACACCCATTTTAAAGGTTGAGGAAACAAGCCCAGAGAGG  
TTAAGGGAGGAGTTCCTGCCACCAAGGAACCTGCTTTAGTGGGGGATAGT  
GAAGAAGACAATAAAAGATAGTAGTTCAGGCCAGGCGGGGTGCTCACGC  
CTGTAATCCTAGCACTTTTGGGAGGCAGAGATGGGAGGATACTTGAATCC  
AGGCATTTGAGACCAGCCTGGGTAACATAGTGAGACCCTATCTCTACAAA  
ACACTTTTAAAAAATGTACACCTGTGGTCCCAGCTACTCTGGAGGCTAAG  
GTGGGAGGATCACTTGATCCTGGGAGGTCAAGGCTGCAG

SEQ ID NO: 100

### Serine Proteinase Inhibitor, Clade H, Member 1

>gi|32454740|ref|NM\_001235.2| Homo sapiens serine (or cysteine) proteinase inhibitor, clade H (heat shock protein 47), member 1, (collagen binding protein 1) (SERPINH1), mRNA | qPCR assay\_on\_demand\_context match [184..208]

TCTTTGGCTTTTTTTGGCGGAGCTGGGGCGCCCTCCGGAAGCGTTTC  
CAACTTTCCAGAAGTTTCTCGGGACGGGCAGGAGGGGGTGGGGACTGCCA



TATATAGATCCCGGAGCAGGGGAGCGGGCTAAGAGTAGAATCGTGTCGCGGCTCGAGAGCGAGATCAGTCCCCGGCGCTAGCCAGCCGACCCAGGCCACCCGTGGTGACGCAAAACCACTTCCTGGCCATGCGCTCCCTCCTGCTTC TCAGCGCCTTCTGCCTCCTGGAGGCGGCCCTGGCCGCCGAGGTGAAGAAA CCTGCAGCCGCAGCAGCTCCTGGCACTGCGGAGAAGTTGAGCCCCAAGGC GGCCACGCTTGCCGAGCGCAGCGCCGGCCTGGCCTTCAGCTTGTACCAGG CCATGGCCAAGGACCAGGCAGTGGAGAACATCCTGGTGTACCCGTGGTG GTGGCCTCGTCGCTAGGGCTCGTGTCTGCTGGGCGGCAAGGCGACCACGGC GTCGCAGGCCAAGGCAGTGCTGAGCGCCGAGCAGCTGCGCGACGAGGAG GTGCACGCCGGCCTGGGCGAGCTGCTGCGCTCACTCAGCAACTCCACGGC GCGCAACGTGACCTGGAAGCTGGGCAGCCGACTGTACGGACCCAGCTCAG TGAGCTTCGCTGATGACTTCGTGCGCAGCAGCAAGCAGCACTACAACCTGC GAGCACTCCAAGATCAACTTCCGCGACAAGCGCAGCGCGCTGCAGTCCAT CAACGAGTGGGCCGCGCAGACCACCGACGGCAAGCTGCCCCGAGGTCACC AAGGACGTGGAGCGCACGGACGGCGCCCTGCTAGTCAACGCCATGTTCTT CAAGCCACACTGGGATGAGAAATTCCACCACAAGATGGTGGACAACCGTG GCTTCATGGTGACTCGGTCCTATACCGTGGGTGTCATGATGATGCACCGGA CAGGCCTCTACAAC TACTACGACGACGAGAAGGAAAAGCTGCAAATCGTG GAGATGCCCCCTGGCCCCACAAGCTCTCCAGCCTCATCATCCTCATGCCCCAT CACGTGGAGCCTCTCGAGCGCCTTGAAAAGCTGCTAACCAAAGAGCAGCT GAAGATCTGGATGGGGAAGATGCAGAAGAAGGCTGTTGCCATCTCCTTGC CCAAGGGTGTGGTGGAGGTGACCCATGACCTGCAGAAACACCTGGCTGGG CTGGGCCTGACTGAGGCCATTGACAAGAACAAGGCCGACTTGTCACGCAT GTCAGGCAAGAAGGACCTGTACCTGGCCAGCGTGTTCCACGCCACCGCCT TTGAGTTGGACACAGATGGCAACCCCTTTGACCAGGACATCTACGGGGCGC GAGGAGCTGCGCAGCCCCAAAGCTGTTCTACGCCGACCACCCCTTCATCTTC CTAGTGCGGGACACCCAAAGCGGCTCCCTGCTATTCAATTGGGCGCCTGGT CCGGCCTAAGGGTGACAAGATGCGAGACGAGTTATAGGGCCTCAGGGTGC ACACAGGATGGCAGGAGGCATCCAAAGGCTCCTGAGACACATGGGTGCT ATTGGGGTTGGGGGGGAGGTGAGGTACCAGCCTTGGATACTCCATGGGGT GGGGGTGGA AAAACAGACCGGGGTTCCCGTGTGCCTGAGCGGACCTTCCC AGCTAGAATTCACCTCCACTTGACATGGGCCCCAGATACCATGATGCTGA GCCCGAAACTCCACATCCTGTGGGACCTGGGCCATAGTCATTCTGCCTGC CCTGAAAGTCCCAGATCAAGCCTGCCTCAATCAGTATTCATATTTATAGCC AGGTACCTTCTCACCTGTGAGACCAAATTGAGCTAGGGGGGTCAGCCAGC CCTCTTCTGACACTAA AACACCTCAGCTGCCTCCCCAGCTCTATCCCAACC TCTCCCAACTATAAACTAGGTGCTGCAGCCCCCTGGGACCAGGCACCCCC AGAATGACCTGGCCGCAGTGAGGCGGATTGAGAAGGAGCTCCCAGGAGG GGCTTCTGGGCAGACTCTGGTCAAGAAGCATCGTGTCTGGCGTTGTGGGG ATGAACTTTTTGTTTGTTCCTTTTTTAGTTCTTCAAAGATAGGGAGG GAAGGGGGAACATGAGCCTTTGTTGCTATCAATCCAAGAACTTATTTGTA CATTTTTTTTTTCAATAAACTTTTCCAATGACATTTTGTGGAGCGTGGA

AAAA

SEQ ID NO: 101

### Serine Proteinase Inhibitor, Clade B, Member 5

>gi|4505788|ref|NM\_002639.1| Homo sapiens serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 5 (SERPINB5), mRNA | qPCR

forward\_primer match [36..56] | qPCR reverse\_primer match [106..86] | qPCR probe match [60..80]

GGCACGAGTTGTGCTCCTCGCTTGCCTGTTCTTTTCCACGCATTTT  
CCAGGATAACTGTGACTCCAGGCCCGCAATGGATGCCCTGCAACTAGCAA  
ATTCGGCTTTTGGCGTTGATCTGTTCAAACAACATATGTGAAAAGGAGCCAC  
TGGGCAATGTCCTCTTCTCTCCAATCTGTCTCTCCACCTCTCTGTCACTTGC  
TCAAGTGGGTGCTAAAGGTGACACTGCAAATGAAATTGGACAGGTTCTTC  
ATTTTGAAAATGTCAAAGATATACCCTTTGGATTTCAAACAGTAACATCGG  
ATGTAAACAAACTTAGTTCCTTTTACTCACTGAAACTAATCAAGCGGCTCT  
ACGTAGACAAATCTCTGAATCTTTCTACAGAGTTCATCAGCTCTACGAAGA  
GACCCTATGCAAAGGAATTGGAAACTGTTGACTTCAAAGATAAATTGGAA  
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CTTTGAGAACATTTTAGCTGACAACAGTGTGAACGACCAGACCAAATCC  
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ACCAGTGCAGATGATGAACATGGAGGCCACGTTCTGTATGGGAAACATTG  
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GCATGTTTCATCCTACTACCCAAGGATGTGGAGGATGAGTCCACAGGCTTG  
GAGAAGATTGAAAAACAACCTCAACTCAGAGTCACTGTACAGTGGACTAA  
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CAAGGGAGTGGCCCTATCAAATGTTATCCACAAAGTGTGCTTAGAAATAA  
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CACAAGGATGAATTGAATGCTGACCATCCCTTTATTTACATCATCAGGCAC  
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TATTCATTATTTGTCAAATTGTCCGGGGTAGTTGGCAGAAATACAGTCTTC  
CACAAAGAAAATTCCTATAAGGAAGATTTGGAAGCTCTTCTTCCCAGCAC  
TATGCTTTCTTCTTTGGGATAGAGAATGTTCCAGACATTCTCGCTTCCCTG  
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TGTTAAGCCCTGGCAGGCAGGTGTTTATTAAATTTCTGAATTTTGGGGATT  
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AGATCTGGGGCAGCAACCTATAAATCAACACCTTAATATGCTGCAACAAA  
ATGTAGAATATTCAGACAAAATGGATACATAAAGACTAAGTAGCCCATAA  
GGGGTCAAAATTTGCTGCCAAATGCGTATGCCACCAACTTACAAAAACAC  
TTCGTTCCGAGAGCTTTTCAGATTGTGGAATGTTGGATAAGGAATTATAGA  
CCTCTAGTAGCTGAAATGCAAGACCCCAAGAGGAAGTTCAGATCTTAATA  
TAAATTCACTTTCATTTTGTATAGCTGTCCCATCTGGTCATGTGGTTGGCAC  
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AGCCGATATCAGAATTTGTGTTGAAGGAACCTGTCTCTTCATCTAATATGA  
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TGATTAAAGTGCTCACGTTACCTTGACACATAGTTTTTTCAGTCTATGGGTT  
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GTTGGGTGGATAAGCTATCCCTGTTGCCGGTTCATGGATTACTTCTCTATA  
AAAAATATATATTTACCAAAAAATTTTGTGACATTCTTCTCCCATCTCTT  
CCTTGACATGCATTGTAAATAGGTTCTTCTTGTGTTCTGAGATTCAATATTGA  
ATTTCTCCTATGCTATTGACAATAAAATATTATTGAACTACC

SEQ ID NO: 102

**Carcinoembryonic Antigen-Related Cell Adhesion Molecule 5**

>gi|11386170|ref|NM\_004363.1| Homo sapiens carcinoembryonic antigen-  
related cell adhesion molecule 5 (CEACAM5), mRNA | qPCR  
assay\_on\_demand\_context match [2128..2152]

CTCAGGGCAGAGGGGAGGAAGGACAGCAGACCAGACAGTCACAGC  
AGCCTTGACAAAACGTTCTGGAAGTCAAGCTCTTCTCCACAGAGGAGGA  
CAGAGCAGACAGCAGAGACCATGGAGTCTCCCTCGGCCCCCTCCCCACAGA  
TGGTGCATCCCCTGGCAGAGGCTCCTGCTCACAGCCTCACTTCTAACCTTC  
TGGAACCCGCCCCACCACTGCCAAGCTCACTATTGAATCCACGCCGTTCAAT  
GTCGCAGAGGGGAAGGAGGTGCTTCTACTTGTCCACAATCTGCCCCAGCA  
TCTTTTGGCTACAGCTGGTACAAAGGTGAAAGAGTGGATGGCAACCGTC  
AAATTATAGGATATGTAATAGGAACTCAACAAGCTACCCAGGGCCCCGCA  
TACAGTGGTCGAGAGATAATATACCCCAATGCATCCCTGCTGATCCAGAA  
CATCATCCAGAATGACACAGGATTCTACACCCTACACGTCATAAAGTCAG  
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CCCAAGCCCTCCATCTCCAGCAACAACCTCCAAACCCGTGGAGGACAAGGA  
TGCTGTGGCCTTCACCTGTGAACCTGAGACTCAGGACGCAACCTACCTGTG  
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AGCTACAAATGTGAAACCCAGAACCCAGTGAGTGCCAGGCGCAGTGATTC  
AGTCATCCTGAATGTCCTCTATGGCCCCGGATGCCCCCACCATTTCCTCT  
AAACACATCTTACAGATCAGGGGAAAATCTGAACCTCTCCTGCCACGCAG  
CCTCTAACCCACCTGCACAGTACTCTTGGTTTGTCAATGGGACTTTCCAGC  
AATCCACCCAAGAGCTCTTTATCCCCAACATCACTGTGAATAATAGTGGAT  
CCTATACGTGCCAAGCCCATAACTCAGACACTGGCCTCAATAGGACCACA  
GTCACGACGATCACAGTCTATGCAGAGCCACCCAAACCCTTCATCACCAG  
CAACAACCTCAACCCCGTGGAGGATGAGGATGCTGTAGCCTTAACCTGTG  
AACCTGAGATTCAGAACACAACCTACCTGTGGTGGGTAAATAATCAGAGC  
CTCCCGGTCAGTCCCAGGCTGCAGCTGTCCAATGACAACAGGACCCTCAC  
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TATGGCCCAGACGACCCCAACCTTCCCCCTCATACACCTATTACCGTCCA  
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 GCAATAATTCCATAGTCAAGAGCATCACAGTCTCTGCATCTGGAACCTTCTC  
 CTGGTCTCTCAGCTGGGGCCACTGTCGGCATCATGATTGGAGTGCTGGTTG  
 GGGTTGCTCTGATATAGCAGCCCTGGTGTAGTTTCTTCATTTTCAGGAAGAC  
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 AATTGCTTCTTTACCAAGGATATTTACAGAAAAGACTCTGACCAGAGATC  
 GAGACCATCCTAGCCAACATCGTGAAACCCCATCTCTACTAAAAATACAA  
 AAATGAGCTGGGCTTGGTGGCGCGCACCTGTAGTCCCAGTTACTCGGGAG  
 GCTGAGGCAGGAGAATCGCTTGAACCCGGGAGGTGGAGATTGCAGTGAG  
 CCCAGATCGCACCACTGCACTCCAGTCTGGCAACAGAGCAAGACTCCATC  
 TCAAAAAGAAAAGAAAAGAAGACTCTGACCTGTACTCTTGAATACAAGTT  
 TCTGATACCACTGCACTGTCTGAGAATTTCCAAAACCTTAATGAACTAACT  
 GACAGCTTCATGAAACTGTCCACCAAGATCAAGCAGAGAAAATAATTAAT  
 TTCATGGGACTAAATGAACTAATGAGGATTGCTGATTCTTTAAATGTCTTG  
 TTTCCAGATTTTCAGGAACTTTTTTTCTTTTAAGCTATCCACTCTTACAGC  
 AATTTGATAAAATATACTTTTGTGAACAAAAATTGAGACATTTACATTTTC  
 TCCCTATGTGGTCGCTCCAGACTTGGGAACTATTCATGAATATTTATATT  
 GTATGGTAATATAGTTATTGCACAAGTTCAATAAAAATCTGCTCTTTGTAT  
 AACAGAAAAA

SEQ ID NO: 103

### Matrix Metalloproteinase 2

>gi|11342665|ref|NM\_004530.1| Homo sapiens matrix metalloproteinase 2  
 (gelatinase A, 72kDa gelatinase, 72kDa type IV collagenase) (MMP2), mRNA |  
 qPCR forward\_primer match [1713..1732] | qPCR reverse\_primer match [1793..1775]  
 | qPCR probe match [1751..1773]

TGTTTCCGCTGCATCCAGACTTCCTCAGGCGGTGGCTGGAGGCTGC  
 GCATCTGGGGCTTTAAACATACAAAGGGATTGCCAGGACCTGCGGCGGCG  
 GCGGCGGCGGCGGGGGCTGGGGCGCGGGGGCCGGACCATGAGCCGCTGA  
 GCCGGGCAAACCCAGGCCACCGAGCCAGCGGACCCTCGGAGCGCAGCC  
 CTGCGCCGCGGACCAGGCTCCAACAGGCGGCGAGGCGGCCACACGCAC  
 CGAGCCAGCGACCCCGGGCGACGCGCGGGGCCAGGGAGCGCTACGATG  
 GAGGCGCTAATGGCCCGGGGCGCGCTCACGGGTCCCCTGAGGGCGCTCTG  
 TCTCCTGGGCTGCCTGCTGAGCCACGCCGCCGCGCCGTCGCCCATCAT  
 CAAGTTCCCCGGCGATGTCGCCCCCAAACGGACAAAGAGTTGGCAGTGC  
 AATACCTGAACACCTTCTATGGCTGCCCAAGGAGAGCTGCAACCTGTTT  
 GTGCTGAAGGACACACTAAAGAAGATGCAGAAGTTCTTTGGACTGCCCA  
 GACAGGTGATCTTGACCAGAATACCATCGAGACCATGCGGAAGCCACGCT  
 GCGGCAACCCAGATGTGGCCAACTACAATTCTTCCCTCGCAAGCCCAAG  
 TGGGACAAGAACCAGATCACATACAGGATCATTGGCTACACACCTGATCT  
 GGACCCAGAGACAGTGGATGATGCCTTTGCTCGTGCCTTCCAAGTCTGGA  
 GCGATGTGACCCCACTGCGGTTTTCTCGAATCCATGATGGAGAGGCAGAC  
 ATCATGATCAACTTTGGCCGCTGGGAGCATGGCGATGGATACCCCTTTGA  
 CGGTAAGGACGGACTCCTGGCTCATGCCTTCGCCCCAGGCACTGGTGTG

GGGGAGACTCCCATTTTGATGACGATGAGCTATGGACCTTGGGAGAAGGC  
CAAGTGGTCCGTGTGAAGTATGGCAACGCCGATGGGGAGTACTGCAAGTT  
CCCCTTCTTGTTCAATGGCAAGGAGTACAACAGCTGCACTGATACTGGCC  
GCAGCGATGGCTTCCTCTGGTGCTCCACCACCTACAACCTTTGAGAAGGAT  
GGCAAGTACGGCTTCTGTCCCCATGAAGCCCTGTTACCATGGGCGGCAA  
CGCTGAAGGACAGCCCTGCAAGTTTCCATTCCGCTTCCAGGGCACATCCTA  
TGACAGCTGCACCACTGAGGGCCGACGCGATGGCTACCGCTGGTGCGGCA  
CCACTGAGGACTACGACCGCGACAAGAAGTATGGCTTCTGCCCTGAGACC  
GCCATGTCCACTGTTGGTGGGAACCTCAGAAGGTGCCCCCTGTGTCTTCCCC  
TTCATTTTCTGGGCAACAAATATGAGAGCTGCACCAGCGCCGGCCGAG  
TGACGGAAAGATGTGGTGTGCGACCACAGCCAACCTACGATGACGACCGCA  
AGTGGGGCTTCTGCCCTGACCAAGGGTACAGCCTGTTCTCTGTGGCAGCC  
CACGAGTTTGGCCACGCCATGGGGCTGGAGCACTCCCAAGACCCTGGGGC  
CCTGATGGCACCCATTTACACCTACACCAAGAACTTCCGTCTGTCCCAGGA  
TGACATCAAGGGCATTTCAGGAGCTCTATGGGGCCTCTCCTGACATTGACCT  
TGGCACCGGCCCCACCCCCACACTGGGCCCTGTCACTCCTGAGATCTGCA  
AACAGGACATTGTATTTGATGGCATCGCTCAGATCCGTGGTGAGATCTTCT  
TCTTCAAGGACCGGTTTCAATTTGGCGGACTGTGACGCCACGTGACAAGCCC  
ATGGGGCCCCCTGCTGGTGGCCACATTCTGGCCTGAGCTCCCGGAAAAGAT  
TGATGCGGTATACGAGGCCCCACAGGAGGAGAAGGCTGTGTTCTTTGCAG  
GGAATGAATACTGGATCTACTCAGCCAGCACCCCTGGAGCGAGGGTACCCC  
AAGCCACTGACCAGCCTGGGACTGCCCCCTGATGTCCAGCGAGTGGATGC  
CGCCTTTAACTGGAGCAAAAACAAGAAGACATACATCTTTGCTGGAGACA  
AATTCTGGAGATACAATGAGGTGAAGAAGAAAATGGATCCTGGCTTTCCC  
AAGCTCATCGCAGATGCCTGGAATGCCATCCCCGATAACCTGGATGCCGT  
CGTGGACCTGCAGGGCGGCGGTACAGCTACTTCTTCAAGGGTGCCTATT  
ACCTGAAGCTGGAGAACCAAAGTCTGAAGAGCGTGAAGTTTGGAAGCATC  
AAATCCGACTGGCTAGGCTGCTGAGCTGGCCCTGGCTCCCACAGGCCCTT  
CCTCTCCACTGCCTTCGATACACCGGGCCTGGAGAACTAGAGAAGGACCC  
GGAGGGGCCTGGCAGCCGTGCCTTCAGCTCTACAGCTAATCAGCATTCTC  
ACTCTACCTGGTAATTTAAGATTCCAGAGAGTGGCTCCTCCCGGTGCCCA  
AGAATAGATGCTGACTGTACTCCTCCCAGGCGCCCCCTTCCCCCTCCAATCC  
CACCAACCCTCAGAGCCACCCCTAAAGAGATCCTTTGATATTTTCAACGCA  
GCCCTGCTTTGGGCTGCCCTGGTGCTGCCACACTTCAGGCTCTTCTCCTTTC  
ACAACCTTCTGTGGCTCACAGAACCCTTGGAGCCAATGGAGACTGTCTCA  
AGAGGGCACTGGTGGCCCGACAGCCTGGCACAGGGCAGTGGGACAGGGC  
ATGGCCAGGTGGCCACTCCAGACCCCTGGCTTTTCACTGCTGGCTGCCTTA  
GAACCTTTCTTACATTAGCAGTTTGTCTTTGTATGCACTTTGTTTTTTCTTT  
GGGTCTTGTTTTTTTTTCCACTTAGAAATTGCATTTCTTGACAGAAGGACT  
CAGGTTGTCTGAAGTCACTGCACAGTGCATCTCAGCCCACATAGTGATGG  
TTCCCCTGTTCACTCTACTTAGCATGTCCCTACCGAGTCTCTTCTCCACTGG  
ATGGAGGAAAACCAAGCCGTGGCTTCCCGCTCAGCCCTCCCTGCCCCCTCT  
TCAACCATTCCCCATGGGAAATGTCAACAAGTATGAATAAAGACACCTAC  
TGAGTGGC

SEQ ID NO: 104

**Proprotein Convertase Subtilisin/Kexin Type 5**

>gi|20336245|ref|NM\_006200.2| Homo sapiens proprotein convertase subtilisin/kexin type 5 (PCSK5), mRNA | qPCR forward\_primer match [2677..2697] | qPCR reverse\_primer match [2821..2801] | qPCR probe match [2737..2765]

CGGAGGGAGCGCTGGGAGCGAGCAAGCGAGCGTTTGGAGCCCGGG  
CCAGCAGAGGGGGCGCCCGGTGCTGCTGTACCGCTCCCGCTGGTTCATC  
TCCGCCGCGCTCGGGGGCCCCGGGAGGAGCGAGACCGAGTCGGAGAGTC  
CGGGAGCCAAGCCGGGCGAAACCCAACTGCGGAGGACGCCCCGCCCCACT  
CAGCCTCCTCCTGCGTCCGAGCCGGGGAGCATCGCCGAGCGCCCCACGGG  
CCGGAGAGCTGGGAGCACAGGTCCCGGCAGCCCCAGGGATGGTCTAGGA  
GCCGGCGTAAGGCTCGCTGCTCTGCTCCCTGCCGGGGCTAGCCGCTCCTG  
CCGATCGCCCGGGGCTGCGAGCTGCGGCGGGCCCGGGGCTGCTCGCCGGGC  
GGCGCAGGCCGGAGAAGTTAGTTGTGCGCGCCCTTAGTGC GCGGAACAG  
CCAGCGAGCGAGGGAGCAGCGAGGCGCCGGGACCATGGGCTGGGGGAGC  
CGCTGCTGCTGCCCGGGACGTTTGGACCTGCTGTGCGTGCTGGCGCTGCTC  
GGGGGCTGCCTGCTCCCCGTGTGTCGGACGCGCGTCTACACCAACCACTG  
GGCAGTCAAAATCGCCGGGGGCTTCCCGGAGGCCAACCCTATCGCCAGCA  
AGTACGGATTTCATCAACATAGGACAGATAGGGGGCCCTGAAGGACTACTAC  
CACTTCTACCATAGCAGGACGATTAAGGTCAGTTATCTCGAGCAGAGG  
GACCCACAGTTTCATTTCAATGGAACCAAAGGTGGAATGGATCCAACAGC  
AAGTGGTAAAAAAGCGGACAAAGAGGGATTATGACTTCAGTCGTGCCCA  
GTCTACCTATTTCAATGATCCCAAGTGGCCCAGCATGTGGTATATGCACTG  
CAGTGACAATACACATCCCTGCCAGTCTGACATGAATATCGAAGGAGCCT  
GGAAGAGAGGCTACACGGGAAAGAACATTGTGGTCACTATCCTGGATGAC  
GGAATTGAGAGAACCCATCCAGATCTGATGCAAACTACGATGCTCTGGC  
AAGTTGCGACGTGAATGGGAATGACTTGGACCCAATGCCTCGTTATGATG  
CAAGCAACGAGAACAAGCATGGGACTCGCTGTGCTGGAGAAGTGGCAGC  
CGCTGCAAAACAATTCGCACTGCACAGTCGGAATTGCTTTCAACGCCAAGA  
TCGGAGGAGTGCGAATGCTGGACGGAGATGTCACGGACATGGTTGAAGC  
AAAATCAGTTAGCTTCAACCCCCAGCACGTGCACATTTACAGCGCCAGCT  
GGGGCCCCGATGATGATGGCAAGACTGTGGACGGACCAAGCCCCCTCACC  
CGGCAAGCCTTTGAAAACGGCGTTAGAATGGGGCGGAGAGGCCTCGGCTC  
TGTGTTTGTGGGCATCTGGAAATGGTGGAAAGGAGCAAAGACCACTGCT  
CCTGTGATGGCTACACCAACAGCATCTACACCATCTCCATCAGCAGCACT  
GCAGAAAGCGGAAAGAAACCTTGGTACCTGGAAGAGTGTTTCATCCACGCT  
GGCCACAACCTACAGCAGCGGGGAGTCCTACGATAAGAAAATCATCTACTA  
CAGATCTGAGGCAGCGTTGCACGGACAACCACACTGGGACGTCAGCCTCA  
GCCCCCATGGCTGCAGGCATCATTGCGCTGGCCCTGGAAGCCAATCCGTTT  
CTGACCTGGAGAGACGTACAGCATGTTATTGTCAGGACTTCCCGTGCGGG  
ACATTTGAACGCTAATGACTGGAAAACCAATGCTGCTGGTTTAAAGGTGA  
GCCATCTTTATGGATTGGACTGATGGACGCAGAAGCCATGGTGATGGAG  
GCAGAGAAGTGGACCACCGTTCCCGGCAGCACGTGTGTGTGGAGAGCAC  
AGACCGACAAATCAAGACAATCCGCCCTAACAGTGCAGTGCAGTCCATCT  
ACAAAGCTTCAGGCTGCTCGGATAACCCCAACCGCCATGTCAACTACCTG  
GAGCACGTCGTTGTGCGCATCACCATACCCACCCAGGAGAGGAGACCT  
GGCCATCTACCTGACCTCGCCCTCTGGAAGTGGTCTCAGCTTTTGGCCAA  
CAGGCTATTTGATCACTCCATGGAAGGATTCAAAAACCTGGGAGTTCATGA  
CCATTCATTGCTGGGGAGAAAGAGCTGCTGGTGAAGTGGGTCCTTGAAGTT

TATGATACTCCCTCTCAGCTAAGGAACTTTAAGACTCCAGGTAAATTGAA  
 AGAATGGTCTTTGGTCCTCTACGGCACCTCCGTGCAGCCATATTCACCAAC  
 CAATGAATTTCCGAAAGTGGAACGGTTCCGCTATAGCCGAGTTGAAGACC  
 CCACAGACGACTATGGCACAGAGGATTATGCAGGTCCCTGCGACCCTGAG  
 TGCAGTGAGGTTGGCTGTGACGGGCCAGGACCAGACCACTGCAATGACTG  
 TTTGCACTACTACTACAAGCTGAAAAACAATACCAGGATCTGTGTCTCCA  
 GCTGCCCCCCTGGCCACTACCACGCCGACAAGAAGCGCTGCAGGAAGTGT  
 GCCCCCAACTGTGAGTCCTGCTTTGGGAGCCATGGTGACCAATGCATGTCC  
 TGCAAATATGGATACTTTCTGAATGAAGAAACCAACAGCTGTGTTACTCA  
 CTGCCCTGATGGGTCATATCAGGATACCAAGAAAAATCTTTGCCGGAAT  
 GCAGTGAAACTGCAAGACATGTACTGAATTCCATAACTGTACAGAATGT  
 AGGGATGGGTAAAGCCTGCAGGGATCCCGGTGCTCTGTCTCCTGTGAAGA  
 TGGACGGTATTTCAACGGCCAGGACTGCCAGCCCTGCCACCGCTTCTGCG  
 CCACTTGTGCTGGGGCAGGAGCTGATGGGTGCATTAACTGCACAGAGGGC  
 TACTTCATGGAGGATGGGAGATGCGTGCAGAGCTGTAGTATCAGCTATTA  
 CTTTGACCACTCTTCAGAGAAATGGATACAAATCCTGCAAAAAATGTGATA  
 TCAGTTGTTTGACGTGCAATGGCCCAGGATTCAAGAACTGTACAAGCTGC  
 CTAGTGGGTATCTCTTAGACTTAGGAATGTGTCAAATGGGAGCCATTTGC  
 AAGGATGCAACGGAAGAGTCCTGGGCGGAAGGAGGCTTCTGTATGCTTGT  
 GAAAAAGAACAATCTGTGCCAACGGAAGGTTCTTCAACAACCTTGCTGCA  
 AAACATGTACATTTCAAGGCTGAGCAGCCATCTTAGATTTCTTTGTTCTG  
 TAGACTTATAGATTATTCCATATTATTA AAAAAGAAAAAAAAAAGCCAAA  
 AG

SEQ ID NO: 105

### Carboxypeptidase N, polypeptide 2, 83kD

>gi|18554966|ref|XM\_087358.1| Homo sapiens carboxypeptidase N, polypeptide 2, 83kD (CPN2), mRNA

ATGGGTTGTGACTGCTTCGTCCAGGAGGTGTTCTGCTCAGATGAGG  
 AGCTTGCCACCGTCCCGCTGGACATCCCGCCATATACGAAAAACATCATC  
 TTTGTGGAGACCTCGTTCACCACATTGGAAACCAGAGCTTTTGGCAGTAAC  
 CCCAACTTGACCAAGGTGGTCTTCCTCAACACTCAGCTCTGCCAGTTTAGG  
 CCGGATGCCTTTGGGGGGCTGCCAGGCTGGAGGACCTGGAGGTACAGG  
 CAGTAGCTTCTTGAACCTCAGCACCAACATCTTCTCCAACCTGACCTCGCT  
 GGGCAAGCTCACCTCAACTTCAACATGCTGGAGGCTCTGCCCCGAGGGTC  
 TTTTCCAGCACCTGGCTGCCCTGGAGTCCCTCCACCTGCAGGGGAACCAGC  
 TCCAGGCCCTGCCAGGAGGCTCTTCCAGCCTCTGACCCATCTGAAGACA  
 CTCAACCTGGCCCAGAACCTCCTGGCCCAGCTCCCGGAGGAGCTGTTCCA  
 CCCACTCACCAGCCTGCAGACCCTGAAGCTGAGCAACAACGCGCTCTCTG  
 GTCTCCCCCAGGGTGTGTTTGGCAAACCTGGGCAGCCTGCAGGAGCTCTTCC  
 TGGACAGCAACAACATCTCGGAGCTGCCCCCTCAGGTGTTCTCCAGCTCT  
 TCTGCCTAGAGAGGCTGTGGCTGCAACGCAACGCCATCACGCACCTGCCG  
 CTCTCCATCTTTGCCTCCCTGGGTAATCTGACCTTTCTGAGCTTGCAGTGG  
 AACATGCTTCGGGTCTGCTGCTGCCGGCCTCTTTGCCACACCCCATGCCTG  
 GTTGGCCTGTCTCTGACCCATAACCAGCTGGAGACTGTCGCTGAGGGCAC  
 CTTTGCCACCTGTCCAACCTGCGTTCCTCATGCTCTCATAACAATGCCATT  
 ACCACCTCCCAGCTGGCATCTTCAGAGACCTGGAGGAGTTGGTCAAACCT  
 CTACCTGGGCAGCAACAACCTTACGGCGCTGCACCCAGCCCTCTTCCAGA  
 ACCTGTCCAAGCTGGAGCTGCTCAGCCTCTCCAAGAACCAGCTGACCACA  
 CTTCCGGAGGGCATCTTCGACACCAACTACAACCTGTTCAACCTGGCCCTG



CACGGTAACCCCTGGCAGTGC GACTGCCACCTGGCCTACCTCTTCAACTGG  
CTGCAGCAGTACACCGATCGGCTCCTGAACATCCAGACCTACTGCGCTGG  
CCCTGCCTACCTCAAAGGCCAGGTGGTGCCCGCCTTGAATGAGAAGCAGC  
TGGTGTGTCCCGTCACCCGGGACCACTTGGGCTTCCAGGTCACGTGGCCG  
GACGAAAGCAAGGCAGGGGGCAGCTGGGATCTGGCTGTGCAGGAAAGGG  
CAGCCCGGAGCCAGTGCACCTACAGCAACCCCGAGGGCACCGTGGTGCTC  
GCCTGTGACCAGGCCAGTGTGCTGGCTGAACGTCCAGCTCTCTCCTTGG  
CAGGGCTCCCTGGGACTGCAGTACAATGCTAGTCAGGAGTGGGACCTGAG  
GTCGAGCTGCGGTTCTCTGCGGCTCACCGTGTCTATCGAGGCTCGGGCAGC  
AGGGCCCTAGTAGCAGCGCATAAGGAGCTGGGGAAGGGGGCTTTGGGG  
CCTGCCCACGCGACAGGTAGGGGCGGAGGGGAGCTGAGTCTCCGAAGCTT  
GGCTTT

SEQ ID NO: 106

#### Hyaluronan and proteoglycan link protein 4

>gi|30794471|ref|NM\_023002.1| Homo sapiens hyaluronan and proteoglycan link protein 4 (HAPLN4), mRNA

CGGGGGCGCGCGGGCAAGATGGTGTGCGCTCGGGCGGCCCTCGG  
TCCCGGCGCGCTCTGGGCCGCGGCCTGGGGCGTCCTGCTGCTCACAGCCC  
CTGCGGGGGCGCAGCGTGGCCGGAAGAAGGTCGTGCACGTGCTGGAGGG  
TGAGTCGGGCTCGGTAGTGGTACAGACAGCGCCTGGGCAGGTGGTAAGCC  
ACCGTGGTGGCACCATCGTCTTGCCCTGCCGCTACCACTATGAGGCAGCC  
GCCCACGGTCACGACGGCGTCCGGCTCAAGTGGACAAAGGTGGTGGACCC  
GCTGGCCTTCACCGACGTCTTCGTGGCACTAGGCCCCAGCACCGGGCATT  
CGGCAGCTACCGTGGGCGGGCTGAGCTGCAGGGCGACGGGCCTGGGGAT  
GCCTCCCTGGTCTCTCCGCAACGTACGCTGCAAGACTACGGGCGCTATGA  
GTGCGAAGTCACCAATGAGCTGGAAGATGACGCTGGCATGGTCAAGCTGG  
ACCTGGAAGGCGTGGTCTTTCCCTACCACCCCGTGGAGGCCGATACAAG  
CTGACCTTCGCGGAGGGCGCAGCGCGCGTGCGCCGAGCAGGACGGCATCCT  
GGCATCTGCAGAACAGCTGCACGCGGCCTGGCGCGACGGCCTGGACTGGT  
GCAACGCGGGCTGGTTGCGCGACGGCTCAGTGCAATACCCCGTGAACCGG  
CCCCGGGAGCCCTGCGGCGGGCCTGGGGGGGACCGGGAGTGCAGGGGGCG  
GCGGTGATGCCAACGGGGGGCCTGCGCAACTACGGGTATCGCCATAACGCC  
GAGGAACGCTACGACGCCTTCTGCTTCACGTCCAACCTGCCGGGGCGCGT  
GTTCTTCTGAAGCCGCTGCGACCTGTACCCTTCTCCGGAGCTGCGCGCGC  
GTGTGCTGCGCGTGGCGCGGCCGTGGCCAAGGTGGGGCAGCTGTTGCGCG  
CGTGGAAAGCTGCAGCTGCTAGACCGCTGCACCGCGGGTTGGCTGGCCGAT  
GGCAGTGC GCGCTACCCCATCGTGAACCCGCGAGCGCGCTGCGGAGGCCG  
CAGGCCTGGTGTGCGCAGCCTCGGCTTCCCGGACGCCACCCGACGGCTCT  
TCGGCGTCTACTGCTACCGCGCTCCAGGAGCACCGGACCCGGCACCTGGC  
GGCTGGGGCTGGGGCTGGGCGGGCGGCGGCGGCTGGGCAGGGGGCGCGC  
GCGATCCTGCTGCCTGGACCCCTCTGCACGTCTAGGCTGGGAGTAGGCGG  
ACAGCCAGGGCGCTTGACCACTGGTCTAGAGCCCTGTGGTCCCCTGGAGC  
CTGGCCACGCCCTTGAAGCCCTGGACACTGGCCACATTCCCTGTGGTCCCT  
TACAAACTAACTGTGCCCTGGGGTCCCTGAAGACTGGCTAGTCCTGGCA  
GAACAGTACTTTGGAGTTCCCTGGAGCCTGGCCAGCCCTACCTCTTCTGG  
ATAGAGGATTCCCCCAACTCCCCAACTTTCTCCATGAGGGTCACGCCCCCT  
GAGGACCTCAGGAGGCCAGCAGAACCCGCAAGGCTCCTGAAGACTGGCCA  
CGCCTCCTGAGACCACTTGGAACAGACCAACTGCCCCCGTGGTGCCTG  
GTGGCTGGACCCCGGGATTGACTAGAGACCGGCCGTACACCTTCTGCAT



CTCACTGGAGACTGAACACTAGTCCCTTGCGGTCACGTGGGACACTGGGC  
 GCCTCCTCCTCCCCCTCCTCCTCACCTGGAGAGACTACAGGAACTTCAGGG  
 TCACTCCCCGTGGTCACATGGAGGTTGTGGGCCGAGGCGCTTATTTTCCCT  
 TATGGTGACCTGAGTCCTGGAGACTCCCATTCTCCCCCTCTCCCTGAGAGT  
 CCCCTGCAGTTTCTGGGTAACAGGGCACACCCCTCTAGTTTCATGGGCGAG  
 CACCCCATCTGCCACCTCAGACTGACACACAGCCAGCTGGCTCACTTACT  
 GGGGGCCACGTCCCACCCCTCAGATATTTCTTTGAAGGGAGAGCAAACCC  
 ACCCTGTCTCTGACGTCCCTTTCCCAACTGTCACCAAACAGACCATCTTC  
 CCAGGCCTGGGGACCGGTAAGATCCATGTCACTAGTTATGCAGAGCAGTT  
 GCCTTGGGTCCCCTGTCAACCAAGGCAACCAGTCCTGCTGCTACCTGTAC  
 CTAGAGTCACACACCCCTTCCCTCATCAGGCACACCCATGAAGACAGTGC  
 CTCCCTCCTCCAGCTGTAACCATGGATACCACACATTTCTCATCTCATTGG  
 CCCCCACCCAGAGACCTCCACCTCAACTTCTGGCTGTCCCTACCCTGACT  
 CACCGCCATGGAGATCACCTCCCCGAAGCTGTCGCCAGGGTGACCCAAC  
 ATCCAGTTCTCCGGCTCTCACCATGGAAACAACTGTCCCTGTCCCCAGGC  
 CCACTCCAGTTCCAGACCACCCCTCCATGCTCCACCCCCAGGCGGTTTGAC  
 CCCACCACTGTTGCCATGGTGACCAAACTCTGGAGTCCGAGGTAACAGAA  
 CACCTGTCCCCCTAGGCTTTTCTTGTGGACAACGGGGCCCTGTTACCAA  
 GCTGTTGCCATAGAGACTGTCAACGTTGTCTCATGACAACAGACTTCCA  
 GTTCTCAGGAAGTTCTCATTGTGGGCCAGAAGTCCTGGGTGCCTCCTACTA  
 GGGCTACCCTACTGCACCCCATCAGGGGCCTGATGGCTGCCCTTCCCCAG  
 ACAGGGCTGGACTTCTGGAGCTGCTAAGCCACCCTCCGTTTGCACGTTAAC  
 TCTATGCCGGATAGCAGCTGTGCACGAGACAATCTTGCAACACCCGGGCA  
 TGTTTGTCTGCTCCTACAAATGAGGAAACCGAGCCTATGGCGTGCCCTG  
 GTCTGTTGAGATATGCAAGCACTGAGCTCCTCTTTTGTCTCTGAGACCC  
 ATCTCCATTCTCACCCAGTTCTCTCTCCTTCCCTGACCCCCACCCACATTT  
 CCCTCCTTAGAGATCCAGGAGGGATGGAATGTTCTTTAAATTCACACC  
 CACCAGGCTCTAAGCGGCGATCTGTGCTAAGAGGTCAGGACCCAGCCGAA  
 GTCCTCGGCGTTGACAGGCAGCTGGGGGGACATGATCCATGGACAAGGCC  
 ATCCCGGCCGTGGGAGACCCAGTCCCGAAGTCTTGCCTGCAGGAGTACT  
 GGGGTCCCCCTGGGGCCCTCTTTACTGTACGTCATCTCTAGGAAACCTAT  
 CTCTGAGTTTGGGACCAGGTCGGTTTGGGTTTGAATTCTGCCTCTTCTTGC  
 TCACTGTGTGACCAAGTGACAACTCCTTCTGAACCTGTGTTCTCCCACTG  
 TACCAGGGCTGTTCTGTGGTCCCCGTGAGTGCCAAGCATACAGTAGGGGC  
 TCAATAAATCCTTGT

SEQ ID NO: 107

### Immunohistochemistry

8uM frozen sections were cut from tissue blocks and mounted onto APES slides. The tissue was then fixed in acetone for 10 minutes before being air-dried. The slides were then soaked in 0.3% hydrogen peroxide in methanol for 10 minutes and washed in phosphate-buffered saline (PBS). Non-specific binding sites were blocked by incubating the slides in 20% serum from the appropriate animal and washing again in PBS. Primary antibody diluted in PBS containing 1% serum was then added to the slides. After incubation for 1 hour, the slides were again washed in PBS before incubating with the secondary antibody for a further 1 hour. After final washing in

PBS, the secondary antibody was detected with diaminobenzidine tetrahydrochloride dissolved in Tris buffered saline (TBS), before being washed in TBS and water. The slides were then counter stained in haematoxylin and viewed under a light microscope.

In certain embodiments, gastric tumors can be localized *in situ* using stains based on cancer markers of this invention. At least one marker may be forming amyloid structures that can be visualized using Congo red or equivalent, non-specific amyloid stains.

### **Tests for Gastric Cancer Markers in Body Fluids**

In several embodiments, assays for GTM can be desirably carried out on samples obtained from blood, plasma, serum, peritoneal fluid obtained for example using peritoneal washes, or other body fluids, such as urine, lymph, cerebrospinal fluid, gastric fluid or stool samples.

In general, methods for assaying for oligonucleotides, proteins and peptides in these fluids are known in the art. Detection of oligonucleotides can be carried out using hybridization methods such as Northern blots, Southern blots or microarray methods, or qPCR. Methods for detecting proteins include such as enzyme linked immunosorbent assays (ELISA), protein chips having antibodies, suspension beads, radioimmunoassay (RIA), Western blotting and lectin binding. However, for purposes of illustration, fluid levels of a GTM can be quantified using a sandwich-type enzyme-linked immunosorbent assay (ELISA). For plasma assays, a 5 uL aliquot of a properly diluted sample or serially diluted standard GTM and 75 uL of peroxidase-conjugated anti-human GTM antibody are added to wells of a microtiter plate. After a 30 minute incubation period at 30°C, the wells are washed with 0.05% Tween 20 in phosphate-buffered saline (PBS) to remove unbound antibody. Bound complexes of GTM and anti-GTM antibody are then incubated with o-phenyldiamine containing H<sub>2</sub>O<sub>2</sub> for 15 minutes at 30°C. The reaction is stopped by adding 1 M H<sub>2</sub>SO<sub>4</sub>, and the absorbance at 492 nm is measured with a microtiter plate reader.

It can be appreciated that anti-GTM antibodies can be monoclonal antibodies or polyclonal antisera. It can also be appreciated that any other body fluid can be suitably studied.

Certain markers are known to be present in plasma or serum. These include osteopontin (Hotte et al., Cancer 95(3): 507-510 (2002)), prostate-specific antigen

(Martin et al., Prostate Cancer Prostatic Dis. (March 9, 2004) (Pub Med No: PMID: 15007379), thyroglobulin (Hall et al., Laryngoscope 113(1):77-81 (2003); Mazzaferri et al., J. Clin. Endocrinol. Metab. 88(4):1433-14421 (2003), matrix metalloproteinase-2 and -9 (Kuo et al., Clin. Chem. Acta. 294(1-2):157-168 (2000), CEA and TIMP1 (Pellegrini et al., Cancer Immunol. Immunother. 49(7):388-394 (2000). Thus, because some of the above markers are also useful markers for GTM, plasma, serum or other fluid assays are already available for their detection and quantification. Because many proteins are either (1) secreted by cells, (2) sloughed from cell membranes, or (3) are lost from cells upon cell death, other GTM are also present in body fluids, such as plasma, serum and the like. Therefore, in embodiments of this invention, detection of GTM in conveniently obtained samples will be useful and desirable and can be a basis for diagnosis of gastric cancer.

#### Western Analysis

Proteins were extracted from gastric tissue using a TriReagent and guanidine HCl extraction method. The non-aqueous phase from the TriReagent extraction of RNA was mixed with 1.5vols of ethanol and centrifuged to remove DNA and OCT medium. 0.5mls of supernatant was mixed with 0.75ml isopropanol, incubated at room temperature for 10 minutes, and then centrifuged. The pellet was washed three times in 1ml 0.3M guanidine HCl in 95% ethanol and once in ethanol alone, then resuspended in 50ul 1% SDS.

Proteins were quantified and electrophoresed on SDS polyacrylamide gels using standard methods. Briefly, the separated proteins were transferred to PVDF membrane using the BioRad trans-blot electrophoretic transfer cell using standard methodology. The membranes were then blocked with a solution containing non-fat milk powder for 30 minutes before being incubated with primary antibody for 2 hours at room temperature. After washing, the membrane was incubated with secondary antibody for 1 hour at room temperature. After final washes, bound antibody was visualized using the ECL detection system (Amersham Biosciences).

Detection of markers in the serum can be accomplished by providing a sample of serum using known methods and then subjecting the serum sample to analysis, either using oligonucleotide probes or antibodies directed against the protein of interest. Immunoblotting, including Western blotting analysis can be especially useful to determine whether alternatively expressed proteins are present in the serum.

Additionally, other body fluids may contain markers, and include peritoneal fluid, cerebrospinal fluid and the like. It is not necessary for a marker to be secreted, in a physiological sense, to be useful. Rather, any mechanism by which a marker protein or gene enters the serum can be effective in producing a detectable, quantifiable level of the marker. Thus, normal secretion of soluble proteins from cells, sloughing of membrane proteins from plasma membranes, secretion of alternatively spliced forms of mRNA or proteins expressed therefrom, cell death (either apoptotic) can produce sufficient levels of the marker to be useful. There is increasing support for the use of serum markers as tools to diagnose and/or evaluate efficacy of therapy for a variety of cancer types.

Yoshikawa et al., (Cancer Letters, 151: 81-86 (2000) describes tissue inhibitor of matrix metalloproteinase-1 in plasma of patients with gastric cancer.

Rudland et al., (Cancer Research 62: 3417-3427 (2002) describes osteopontin as a metastasis associated protein in human breast cancer.

Buckhaults et al., (Cancer Research 61:6996-7001 (2002) describes certain secreted and cell surface genes expressed in colorectal tumors.

Kim et al., (JAMA 287(13):1671-1679 (2002) describes osteopontin as a potential diagnostic biomarker for ovarian cancer.

Hotte et al., (AJ. American Cancer Society 95(3):507-512 (2002) describes plasma osteopontin as a protein detectable in human body fluids and is associated with certain malignancies.

Martin et al., (Prostate Cancer Prostatic Dis. March 9, 2004 (PMID: 15007379) (Abstract) described use of human kallikrein 2, prostate-specific antigen (PSA) and free PSA as markers for detection of prostate cancer.

Hall et al (Laryngoscope 113(1):77-81 (2003) (PMID: 12679418) (Abstract) described predictive value of serum thyroglobulin in thyroid cancer.

Mazzaferri et al., (J. Clin. Endocrinol. Metab. 88(4):1433-1441 (2003) (Abstract) describes thyroglobulin as a potential monitoring method for patients with thyroid carcinoma.

Whitley et al, (Clin. Lab. Med. 24(1):29-47 (2004) (Abstract) describes thyroglobulin as a serum marker for thyroid carcinoma.

Kuo et al (Clin. Chim. Acta. 294(1-2):157-168 (2000) (Abstract) describes serum matrix metalloproteinase-2 and -9 in HCV- and HBV-infected patients.

Koopman et al., (Cancer Epidemiol. Biomarkers Prev 13(3):487-491 (2004) (Abstract) describes osteopontin as a biomarker for pancreatic adenocarcinoma.

Pellegrini et al., (Cancer Immunol. Immunother. 49(7):388-394 (2000) (Abstract) describes measurement of soluble carcinoembryonic antigen and TIMP1 as markers for pre-invasive colorectal cancer.

Thus, we have identified numerous genes and/or proteins that are useful for developing reagents, devices and kits for detecting and evaluating gastric cancer. One or more markers of gastric can be used, either singly or in combination to provide a reliable molecular test for gastric cancer.

### EXAMPLES

The examples described herein are for purposes of illustrating embodiments of the invention. Other embodiments, methods and types of analyses are within the scope of persons of ordinary skill in the molecular diagnostic arts and need not be described in detail hereon. Other embodiments within the scope of the art are considered to be part of this invention.

#### **Example 1: Identification of Markers for Gastric Malignancy**

Figure 2 depicts a table that shows results of studies using 38 markers for gastric malignancy selected using the above criteria. The Figure 2 includes the symbol for the gene ("symbol"), the MWG oligo number, the NCBI mRNA reference sequence number, the protein reference sequence number, the fold change between tumor and non-tumor gene expression, the fold change rank relative to other genes in the microarray analysis, the results of an original, unadjusted Student's t-test, the results of the Bonferroni-adjusted p value and the results of the 2-sample Wilcoxon test.

The median fold change (tumor: non malignant tissue) for these 34 genes ranged from 1.6 to 7 and the median change in fold change rank ranged from -16,995 to -25,783. The maximum possible change in fold change rank was -29,718. For each of the markers shown, the statistical significance of their specificity as cancer markers was found to be extremely high. The Bonferroni-adjusted p values were, in general, all below  $10^{-6}$  or less, indicating that diagnosis using these markers is very highly associated with gastric cancer.

The three cystatins (CST1, CST2, and CST4) are highly homologous and represented by the same oligonucleotide on the microarray and unless otherwise stated, are referred to collectively as "CST1,2,4."

All proteins depicted in Figure 2 were predicted to have signal peptides using the SMART package (European Molecular Biology Laboratory). The signal peptides are known to target synthesized proteins to the extracellular compartment and can therefore be secreted into the interstitial fluid, from which they can have access to the blood. In fact, some proteins of this invention have been detected in serum.

Each of the genes depicted in Figure 2 exhibited a change in intensity rank greater than the two oligonucleotides on the array corresponding to CEA, the marker most frequently used in clinical practice to monitor gastric cancer progression.

#### **Example 2: qPCR Analysis**

More sensitive and accurate quantitation of gene expression was obtained for a subset of the genes shown in Figure 3 using qPCR. RNA from 46 tumor and 49 non-malignant samples was analyzed for 23 genes identified by the microarray analysis (Figure 2) and results are shown in Figure 3. Figure 3 includes the gene symbol, median fold change between cancer and normal tissue, and the % of tumor samples with expression levels greater than the 95<sup>th</sup> percentile of expression levels in non-malignant samples. 12 tumor samples and 9 normal samples were excluded from the analysis because of high (>75%) normal cell contamination, a high degree of necrosis (>40%), or poor hybridization signal on the microarrays. The median fold change (tumor tissues compared to the median non-malignant tissue expression) for these 23 genes ranged from 3 to 525 fold (Figure 3).

The level of expression of genes ASPN, CST1,2,4, LOXL2, TIMP1, SPP1, SFRP4, INHBA, THBS2 and SPARC was greater in tumors than the 95<sup>th</sup> percentile of the non-malignant range for  $\geq 90\%$  of cases (Figure 3). For the remainder of genes, the expression in tumors was greater than the 95<sup>th</sup> percentile in >50% of samples. Each tumor over-expressed at least seven genes greater than the 95<sup>th</sup> percentile indicating that combinations of markers will lead to comprehensive coverage of all gastric tumors.

**Example 3: Validation of Array Data Using qPCR**

Array data was validated using quantitative, real-time PCR (qPCR) on the tumor and non-malignant samples with probes for 24 genes. Of all 24 genes studied, 20 showed a strong correlation between the two techniques. Four of these analyses are shown in Figures 4a – 4d, which depict graphs of the relative expression for the 4 selected cancer markers detected using array and qPCR methods. For each graph in Figure 4, the horizontal axis represents the array log<sub>2</sub> fold change in gene expression, and the vertical axis represents the qPCR log<sub>2</sub> fold change in gene expression. We found that there was a strong correlation between the two methods, as indicated by the co-variant relationship between the methods. The strong correlation indicates that both microarray fold change analysis and qPCR are suitable methods for detecting changes in the expression of gastric cancer marker genes and therefore can be used as an accurate, sensitive screening method. It can also be appreciated from Figures 4a – 4d that qPCR can be more sensitive at detecting changes in expression than are array methods. Thus, in situations in which early detection is especially desirable, qPCR may be especially useful.

Figures 5a – 5w depict histograms comparing frequency of observation of expression of each of a series of 23 genes (vertical axis) and the log<sub>2</sub> fold change in expression for that gene (horizontal axis), for both normal tissue (open bars) and tumor tissues (black bars). We found surprisingly that for each of these 23 genes, there was substantial separation in the frequency distributions between normal and tumor tissue, as reflected by the low degree of overlap between the frequency distribution curves. For example, Figure 5b depicts the results for CST 1, 2, 4, for which there was only one normal sample observed to have an expression level in the tumor range. In other cases (e.g., Figure 5n; for PRS11) each frequency distribution curve was relatively narrow and there was a degree of overlap. However, even for this marker, the median log<sub>2</sub> fold change showed a substantial separation of the amount of gene expression. In other cases, (e.g., Figure 5a; ASPN), although there was some overlap, there was a clear separation of the median log<sub>2</sub> fold expression between normal and tumor samples.

Figure 6 depicts a histogram of the number of genes exhibiting a significantly increased expression (“over-expression”) in tumor samples compared to normal samples (vertical axis) and the individual samples tested. In each case, the tumor sample exhibited multiple genes with elevated expression levels. The lowest number

of genes having increased expression was 7, found in sample E123. This finding indicates that, in situations in which multiple genes are over-expressed relative to normal tissue, the reliability of cancer detection can be very high, making diagnosis of cancer more certain. However, in some cases, elevation of expression of a single marker gene is sufficient to lead to the diagnosis of cancer.

Our previous comparison with the serum marker most frequently used currently for detection of gastric cancer, CEA, was based on difference in intensity rank of array data between tumors and normal samples. This comparison was verified using qPCR data for the markers and CEA.

Figures 7a-7c depict graphs of the relative log<sub>2</sub> expression (compared to a reference RNA preparation) of markers in individual tumor samples and non-malignant samples compared to the expression of the gene for the tumor marker, CEA. CEA is the serum marker currently most used to monitor progression of gastric cancer. The zero point is defined to be the median normal expression for each marker. It can be seen that there is extensive overlap between the expression of the CEA gene (CEACAM5) in tumor samples and normal samples. This overlap is markedly less in the gastric cancer markers ASPN, CSPG2, CST1,2,4, IGFBP7, INHBA, LOXL2, LUM, SFRP4, SPARC, SPP1, THBS2, TIMP1, adican, LEPRE1, and EFEMP2. For the other markers in Figures 7b-7c, ASAH1, SFRP2, GGH, MMP12, KLK10, TG, PRSS11 and TGFB1, the overlap between the tumor expression range and the non-malignant tissue expression range is greater than the overlap for the above markers, but still less than that of CEA, indicating that all of the herein described new markers are quantitatively better than CEA, and therefore can provide more reliable diagnosis.

To minimize effects of variable tissue handling, tumor:normal (non-malignant) fold changes were calculated using qPCR data from tumor and non-malignant tissue samples derived from the same patient. Such paired analysis corrects for differences in background levels of gene expression in different individuals and minimizes the effects of tissue handling on RNA quality. For example, if the resected stomach was at room temperature for an hour, the transcripts from the normal and tumor samples will be degraded to the same extent.

Figure 8 summarizes the T:N expression levels determined by qPCR for the markers, but used paired data (i.e., tumor and non-malignant samples) from the same individual. Figure 8 also includes expression data for six genes that were not included in Figure 3. The additionally studied genes are MMP2, CGR11, TGFB1, PCSK5,



SERPINB5, and SERPINH1. Identifying information and probes are shown in Figures 1 and 2. Figure 8 shows the median T:N fold change and the maximum T:N fold change for 29 gastric cancer markers in these 40 patients with "paired" samples. 27 of the 29 markers have a median T:N difference greater than or equal to the prior art marker, CEA. 29/29 of the markers have a higher percentage of paired samples in which the expression in the tumor sample exceeds the expression in the normal sample.

Figures 9a – 9d depict scatter dot plots of data from tumor and normal tissue from the same individuals. Each point represents the fold-change, within patient, in expression of the markers in tumor tissue relative to the expression in non-malignant tissue. All of the markers studied have better discrimination of tumor from non-tumor tissue than CEA. Three markers, CST1,2,4, ASPN and SFRP4 showed 100% discrimination between the paired tumor and normal samples. That is, for those markers, every tumor tissue had greater expression than did the corresponding non-tumor tissue from the same individual. In many other markers, for example, Adican, CSPG2, EFEMP2, IGFBP7, INHBA, LOXL2, LUM, SERPINH1, SPARC, SPP1, TGFb1, THBS2 and TIMP1, each had only 2 or 3 individual points for which tumor tissue expression was less than that of the non-tumor tissue. Thus, for those markers, the likelihood that any one pair of tumor and non-tumor tissue would produce a false negative is relatively low (e.g., 3 of 40 or 7.5%; 2 of 40 or 5%, 1 of 40 or 2.5%). Thus, even if the other markers listed immediately above were used, use of multiple samples from an individual patient would produce reliable diagnostic information.

The gene sequences of these markers, and the location of the primers and probes used to detect them, are shown herein above.

To determine if over-expression of the marker genes is independent of the stage of the gastric tumors, the paired T:N log2 fold changes were plotted against the tumor stage (Figures 10a – 10ad). No stage dependency of expression on tumor stage was observed for 26 of the markers listed in Figure 8. These markers were similarly over-expressed in early stage as well as late stage tumors. However, KLK10 showed more consistent over-expression in stage 1 and stage 2 tumors, and PCSK5 and SERPINB5 showed more consistent over-expression in stage 4 tumors. KLK10, PCSK5 and SERPINB5 therefore can be used in determining the stage of gastric tumors.

In a similar analysis, paired T:N log<sub>2</sub> fold changes were plotted against the Lauren classification of the tumor (either diffuse type or intestinal type). Figures 11a – 11ad show that each of the 29 GTMs discriminated between tumor and non-tumor tissue, regardless of whether the type of tumor was intestinal (I) or diffuse (D).

#### **Example 4: Use of Multiple Markers**

As described above, certain markers exhibit an ability to discriminate tumor from non-tumor tissue in 100% of the samples. Other markers, also described above, can be used in combination to achieve very high degrees of discrimination of tumor tissue from non-tumor tissue. Figure 12 depicts a 3-dimensional plot of the expression of 3 markers, SERPINH1, CST1,2,4 and INHBA, expressed as log<sub>2</sub> T:N fold changes for a series of gastric tumor samples and non-malignant gastric samples. There is complete separation between the two groups of samples.

The reliability of successful discrimination of tumor and non-tumor samples using marker combinations is further illustrated by a statistical analysis summarized in Figure 13. This analysis compared the normal distributions of data generated using the qPCR gene expression from paired tumor and non-malignant samples, shows the effect of increasing the numbers of markers used to discriminate between tumor and non-malignant samples on test sensitivity (with a fixed specificity of 95%). Although few of the 29 markers (as shown in Figure 8) have a sensitivity of greater than 90, 95, or 99% when used alone in this analysis, the combination of two or three markers enabled high sensitivity to be reached with large numbers of combinations. For example, 50 combinations of three markers would discriminate between tumor and non-malignant samples with a sensitivity of  $\geq 99\%$  and specificity of  $\geq 95\%$ .

#### **Example 5: Detection of Gastric Tumor Marker Proteins**

In yet further embodiments, GTM proteins can be detected as a basis for diagnosis. In certain situations, the concentration of mRNA in a particular sample, such as a sample containing no cells, it may be difficult to use either microarray or qPCR methods to detect elevations in gene expression. Thus, in certain embodiments, detection of GTM proteins can be accomplished using antibodies directed against either the entire protein, a fragment of the protein (peptide) or the protein core. Methods for detecting and quantifying expression of proteins and peptides are known in the art and can include methods relying on specific antibodies raised against the

protein or peptide. Monoclonal antibodies and polyclonal antisera can be made using methods that are well known in the art and need not be described herein further.

To demonstrate that GTM proteins can be used to discriminate tumor from non-tumor tissue, commercial antibodies were obtained against SPARC (R&D Systems; cat # AF941), THBS2 (Santa Cruz Biotechnology Inc; cat # sc-7655), CSPG2 (Calbiochem; cat # 428060) and IGFBP7 (R&D Systems; cat # AF1334). An additional polyclonal antibody was raised in rabbits (Alpha Diagnostic International Inc; San Antonio) against the cystatin SN peptide sequence 50-66 (C) FAISEYNKATKDDYYRR. SEQ ID NO: 108.

These antibodies were used in either immunohistochemistry or Western analysis of tumor and non-malignant gastric tissue. Each of these markers showed strong tumor:normal differences at the protein level. This confirmed that the over-expression observed at the RNA level for these genes also occurred at the protein level.

Figure 14 shows a Western blot analyses of total protein extracted from two pairs of tumor and non-malignant tissues using antibodies against the proteins encoded by SPARC, CST1 (cystatin SN), IGFBP7 and THBS2. For each marker, the signal is significantly higher in the tumor samples than the non-malignant samples.

The antibody raised against cystatin SN detected three major bands, corresponding to molecular weights of approximately 34, 45 and 65kDa respectively. The lowest molecular weight band is shown in Figure 14. The protein species were larger than the control cystatin SN protein, suggesting that the protein produced by tumors has undergone post-translational modifications or multimerization. Regardless of the mechanism responsible for the differences in molecular weights of CST proteins, Figure 14 demonstrated that CST expression was low in the non-tumor tissue, but was easily observed in blots of tumor-derived proteins.

Figure 14 also showed that SPARC protein is expressed substantially to a greater degree in tumor tissue than in non-tumor tissue. The SPARC protein had gel mobility slower than the form of this protein that was detected in serum (Figure 15), also indicating the occurrence of different post-translational modifications in proteins produced by malignant gastric cells. Regardless of the mechanism(s) responsible for any such modification, the finding that SPARC is over-expressed in tumor tissue relative to non-malignant tissue indicates that SPARC is a useful protein marker.

Similarly, IGFBP7 and THBS2 show over-expression in tumor tissue relative to non-malignant tissue.

Immunohistochemical analysis of tumor and non-malignant tissue was carried out using antibodies against the proteins encoded by CSPG2 (versican) and CST1 (cystatin SN). Immunohistochemical analysis of tissue with antibodies against versican identified strong staining in the extracellular matrix of tumor tissue, but not non-malignant tissue. With the anti-cystatin SN antibodies, strong staining was observed in the area around the outside of the tumor cells. In non-malignant cells, the staining with this antibody was weaker, and observed only on the mucosal surface of the tissue and the lining of the gastric pits. This demonstrated that in non-malignant cells, cystatin SN protein is directed out of the cell onto the mucosal surface and not into the extracellular spaces. Therefore, not only is the cystatin SN protein being produced in higher amounts in tumor tissue than non-malignant tissue, but, unlike the protein produced by the non-malignant tissue, the tumor cystatin SN is in direct contact with the tissue vasculature. To extend these observations, cystatin SN was immunoprecipitated from the supernatant of the gastric cancer cell line, AGS with a monoclonal antibody (R&D Systems; cat # MAB1285) (Figure 16). Large amounts of cystatin SN were detected in the supernatant, confirming that this protein is produced by, and secreted from, gastric epithelial cells.

#### **Example 6: Analysis of Tumor Markers in Serum**

For a marker to be useful for rapid screening, it is desirable for the marker to be present in the serum in sufficient levels for detection. Certain proteins described in Figure 8 can be secreted into the blood at detectable levels from gastric cancers. One marker known to be secreted from gastric tumors into blood in detectable levels is TIMP1. However, if a protein is secreted or shed from any surface of a cell other than a mucosal surface, it will have contact with the interstitial fluid. From there, it can pass either directly into the blood supply through a capillary or via the lymph system. Thus, any shed GTM will be present in blood. Osteopontin, thyroglobulin, and members of the MMP and kallikrein families have previously been described to be elevated in the serum of patients with a range of epithelial cancers, but not gastric cancer. TIMP1 has, however, previously been observed to be elevated in the serum of gastric cancer patients. These findings suggest that the selection criteria for markers in this study, namely over-expression of secreted proteins in tumor tissue but not non-

malignant tissue, can be effectively used to detect markers in the serum, and thus can be of substantial use clinically, without the need for tissue or organ biopsies.

From Figure 15, it is apparent that the serum SPARC has a different molecular weight (depicted here in the Western blot) with the tumor SPARC having a lower molecular weight than the SPARC produced by blood cells. Thus, even though SPARC is produced by tumor and non-tumor blood cells, the presence of tumor SPARC can be determined using molecular size, such as determined using Western analysis, or with an antibody specific for the glycosylated protein produced by the tumor cells.

In another study, we detected cystatin SN in the supernatant of a gastric cancer cell line, AGS. Figure 16 depicts a Western analysis of media alone or a supernatant from AGS cells in culture. The right hand lane of Figure 16 shows a dense band corresponding to cystatin SN protein.

Thus, we conclude from Figure 10 that GTM of this invention are suitable for diagnosing gastric cancers at early, middle or late stages of progression of the disease.

Although certain marker proteins can be glycosylated, variations in the pattern of glycosylation can, in certain circumstances, lead to mis-detection of forms of GTMs that lack usual glycosylation patterns. Thus, in certain embodiments of this invention, GTM immunogens can include deglycosylated GTM or deglycosylated GTM fragments. Deglycosylation can be accomplished using one or more glycosidases known in the art. Alternatively, GTM cDNA can be expressed in glycosylation-deficient cell lines, such as prokaryotic cell lines, including *E. coli*, thereby producing non-glycosylated proteins or peptides. It can also be appreciated that the level and quality of glycosylation can be sensitive to the presence of essential precursors for sugar side-chains. Thus, in the absence of an essential sugar, "normal" glycosylation may not occur, but rather, shorter or missing side chain sugars may be found. Such "glycosylation variants" can be used as immunogens to produce antibodies specific for different types of marker genes.

Additionally, certain GTMs may form homo-or heterodimers or other types of multimeric forms. For example, inhibin beta A is a 47 kDa protein that can form homodimers of 97 kDa molecular weight (activin A) and 92 kDa heterodimers with the 45 kDa protein inhibin beta B (the heterodimers are known as activin AB). Thus, it can be appreciated that Western analysis or other type of assay that provides molecular weight need not be limited to only detection of a monomeric form of a

GTM. Rather, one can readily appreciate that any form of a GTM can be detected, regardless of the molecular weight. Thus, detection of a multimeric form of a GTM can be readily used to diagnose the presence of gastric cancer. Further, for those GTM that are selective for stage (1-4) or type of gastric tumor (diffuse or intestinal), detection of a multimeric form can provide suitable target for evaluating stage or type of gastric cancer.

Once an antibody or antiserum against a GTM is produced, such antibody preparations can be used for in a variety of ways. First, enzyme-linked immunosorbent assay (ELISA) or radioimmunoassay (RIA) methods can be used to quantify GTM proteins or peptides. Immunodetection can be accomplished in tissue samples using immunohistochemistry. These methods are all known in the art and need not be described further herein.

#### **Example 7: Vectors Containing GTM Oligonucleotides**

Other embodiments of this invention include vectors useful for *in vitro* expression of marker genes or portions thereof ("marker peptides") or fragments of marker gene products. For example, vectors can be made having oligonucleotides for encoding GTMs therein. Many such vectors can be based on standard vectors known in the art. This invention also includes vectors that can be used to transfect a variety of cell lines to prepare GTM-producing cell lines, which can be used to produce desired quantities of GTMs for development of specific antibodies or other reagents for detection of GTMs or for standardizing developed assays for GTMs.

It is to be understood that to manufacture such vectors, an oligonucleotide containing the entire open reading frame or a portion of such an open reading frame encoding a portion of the protein to be expressed can be inserted into a vector containing a promoter region, one or more enhancer regions operably linked to the oligonucleotide sequence, with an initiation codon, an open reading frame, and a stop codon. Methods for producing expression vectors are known in the art and need not be repeated herein.

It can also be appreciated that one or more selectable markers can be inserted into an expression vector to permit the expansion of cell lines selected to contain the expression vector of interest. Moreover, one can also insert leader sequences known in the art, in frame, to direct secretion, internal storage or membrane insertion of the protein or protein fragment in the expressing cell.

**Example 8: Cells Transfected with GTM-Containing Vectors**

In still further embodiments, cells are provided that can express GTMs, GTM fragments or peptide markers. Both prokaryotic and eukaryotic cells can be so used. For example, *E. coli* (a prokaryotic cell) can be used to produce large quantities of GTMs lacking in mature glycosylation (if the particular GTM normally is glycosylated). COS cells, 293 cells and a variety of other eukaryotic cells can be used to produce GTMs that are glycosylated, or have proper folding and therefore, three-dimensional structure of the native form of the GTM protein. Methods for transfecting such cells are known in the art and need not be described further herein.

**Example 9: Kits**

Based on the discoveries of this invention, several types of test kits can be produced. First, kits can be made that have a detection device pre-loaded with a detection molecule (or "capture reagent"). In embodiments for detection of GTM mRNA, such devices can comprise a substrate (e.g., glass, silicon, quartz, metal, etc) on which oligonucleotides as capture reagents that hybridize with the mRNA to be detected. In some embodiments, direct detection of mRNA can be accomplished by hybridizing mRNA (labeled with cy3, cy5, radiolabel or other label) to the oligonucleotides on the substrate. In other embodiments, detection of mRNA can be accomplished by first making complementary DNA (cDNA) to the desired mRNA. Then, labeled cDNA can be hybridized to the oligonucleotides on the substrate and detected.

Regardless of the detection method employed, comparison of test GTM expression with a standard measure of expression is desirable. For example, RNA expression can be standardized to total cellular DNA, to expression of constitutively expressed RNAs (for example, ribosomal RNA) or to other relatively constant markers.

Antibodies can also be used in kits as capture reagents. In some embodiments, a substrate (e.g., a multiwell plate) can have a specific GTM capture reagent attached thereto. In some embodiments, a kit can have a blocking reagent included. Blocking reagents can be used to reduce non-specific binding. For example, non-specific oligonucleotide binding can be reduced using excess DNA from any convenient source that does not contain GTM oligonucleotides, such as salmon sperm DNA.

Non-specific antibody binding can be reduced using an excess of a blocking protein such as serum albumin. It can be appreciated that numerous methods for detecting oligonucleotides and proteins are known in the art, and any strategy that can specifically detect GTM associated molecules can be used and be considered within the scope of this invention.

In embodiments relying upon antibody detection, GTM proteins or peptides can be expressed on a per cell basis, or on the basis of total cellular, tissue, or fluid protein, fluid volume, tissue mass (weight). Additionally, GTM in serum can be expressed on the basis of a relatively high-abundance serum protein such as albumin.

In addition to a substrate, a test kit can comprise capture reagents (such as probes), washing solutions (e.g., SSC, other salts, buffers, detergents and the like), as well as detection moieties (e.g., cy3, cy5, radiolabels, and the like). Kits can also include instructions for use and a package.

Although this invention is described with reference to specific embodiments thereof, it can be appreciated that other embodiments involving the use of the disclosed markers can be used without departing from the scope of this invention.

#### INDUSTRIAL APPLICABILITY

Methods for detecting GTM family members include detection of nucleic acids using microarray and/or real time PCR methods and detection of proteins and peptides. The compositions and methods of this invention are useful in the manufacture of diagnostic devices and kits, diagnosis of disease, evaluating efficacy of therapy, and for producing reagents suitable for measuring expression of GTM family members in biological samples.